Louisiana State University
LSU Digital Commons

LSU Historical Dissertations and Theses | Graduate School

1977

Rapid Analytical Procedures and Varying Preservation Conditions for Assessing the Bacterial Quality of Oysters (Crassostrea Virginica).

Neal Duane Singletary
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation
Singletary, Neal Duane, "Rapid Analytical Procedures and Varying Preservation Conditions for Assessing the Bacterial Quality of Oysters (Crassostrea Virginica)." (1977). *LSU Historical Dissertations and Theses*. 3136.
https://digitalcommons.lsu.edu/gradschool_disstheses/3136

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

University Microfilms International
300 North Zeeb Road
Ann Arbor, Michigan 48106 USA
St. John's Road, Tyler's Green
High Wycombe, Bucks, England HP10 8HR

77-28,701

SINGLETARY, Neal Duane, 1950-
RAPID ANALYTICAL PROCEDURES AND VARYING PRESERVATION CONDITIONS FOR ASSESSING THE BACTERIAL QUALITY OF OYSTERS (Crassostrea virginica).

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1977
Microbiology.

Xerox University Microfilms, Ann Arbor, Michigan 48106

© 1977

NEAL DUANE SINGLETARY

ALL RIGHTS RESERVED

RAPID ANALYTICAL PROCEDURES AND VARYING PRESERVATION CONDITIONS FOR ASSESSING THE BACTERIAL QUALITY OF OYSTERS (*Crassostrea virginica*)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agriculture and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
Neal D. Singletary
B.S., Southeastern Louisiana University, 1972
M.S., Southeastern Louisiana University, 1974
August, 1977

ACKNOWLEDGMENT

The author wishes to express sincere gratitude to his major professor, Dr. Robert M. Grodner, for his aid and guidance throughout this investigation.

He wishes to express thanks to Dr. Arthur F. Novak, Head of the Department of Food Science, Dr. R. M. Rao, Dr. James E. Rutledge and Dr. William F. McKnight for serving on his examination committee.

Sincere gratitude is also expressed to Dr. William H. James of the Department of Food Science for his assistance in the statistical analysis involved in this study and for serving on his examination committee.

Appreciation is expressed to Dr. Kenneth L. Koonce of the Department of Experimental Statistics for his assistance in the statistical analysis involved in this study.

TO KAREN

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Oysters deteriorate rapidly; therefore, it is imperative to determine their bacteriological quality within a short period of time prior to processing and packing for commercial distribution. Methods have been developed for assessing the bacterial quality of fresh shucked oysters which require a maximum of 24 hours. Their use in the oyster industry could prevent considerable financial losses by eliminating unacceptable products.

Rapid analytical procedures designed to enumerate aerobic plate count (APC), total coliforms, Escherichia coli (E. coli) and coagulase-positive Staphylococcus aureus were developed and compared to Association of Official Analytical Chemists (AOAC) methods. From September, 1975 through April, 1976, oysters were received at the Louisiana State University (LSU) Food Science laboratory and analyzed by both of these methods. In addition, the effect of 4 major causes of variation in bacterial quality of oysters were demonstrated, namely 2 washing treatments, 2 levels of storage temperature, 3 levels of elapsed storage time and 8 monthly collections.

The rapid APC procedure was a 24-hour plate count technique using Eugon agar. No significant differnce was found between the rapid and official techniques for the APC associated with oysters analyzed throughout iced (0°C) storage. Also, no significant difference was found for the

bivariate interaction washing treatment x laboratory methodology. Significant differences ($p < 0.01$) were found between washing treatments, between harvest months and between elapsed storage times.

The rapid procedure for total coliforms was a 24-hour Lauryl Sulfate Tryptose broth (LST) Most Probable Number (MPN). No significant difference was found between the rapid and official techniques for total coliforms associated with oysters analyzed throughout frozen (-20°C) storage. Also, no significant difference was found for the bivariate interaction washing treatment x laboratory methodology. Significant differences ($p < 0.01$) were found between washing treatments, between harvest months and between elapsed storage times.

No significant difference was found between the 24-hour LST MPN at 44.5°C and the official technique for E. coli associated with oysters analyzed throughout iced and frozen stroages. Also, no significant difference was found for the bivariate interaction washing treatment x laboratory methodology. Significant differences ($p < 0.01$) were found between washing treatments, between harvest months, and between elapsed storage times.

The rapid procedure for coagulase-positive staphylococci was a 24-hour MPN procedure in Tryptic Mannitol Meat broth (TMM) at 37°C. No significant difference between the rapid and official techniques was found for coagulase-positive staphylococci associated with oysters analyzed

throughout iced and frozen storages. Also, no significant difference was found for the bivariate interaction washing treatment x laboratory methodology. Significant differences ($p < 0.01$) were found between harvest months and between elapsed storage times. Significant differences were found between washing treatments throughout iced storage ($p < 0.01$) and throughout frozen storage ($p < 0.05$).

The 24-hour analytical procedures tested in this investigation were found dependable and permit the rapid assessment of bacterial quality of oysters. Oysters now could be bacteriologically examined more often, thus revealing their bacteriological profile as they leave processing plants for distribution and eliminating financial losses in the industry.

INTRODUCTION

Oysters deteriorate rapidly; therefore, it is imperative to determine their bacteriological quality within a short period of time prior to processing and packing for commercial distribution. Methods have been developed for assessing the bacterial quality of fresh shucked oysters which require a maximum of 24 hours. Their use in the oyster industry could prevent considerable financial losses by eliminating unacceptable products.

Rapid methods have been developed for the quality tests aerobic plate count (APC), total coliforms, Escherichia coli (E. coli) and coagulase-positive staphylococci. However, the time required for these analyses vary. Winter et al. (1971) developed rapid methods requiring 4 hours whereby microcolonies were counted on membrane filters. These methods are rather expensive. Rapid procedures for total coliforms and E. coli using labeled lactose require only 1 hour (Levine et al., 1957). A 24-hour technique has been described (U.S. Dept. of Health, Education and Welfare, 1969) for coagulase-positive staphylococci. However, the procedure is not recommended for samples containing less than 100 staphylococci per gram. The purpose of this investigation is to determine the reliability of rapid methods developed in this laboratory for the assessment of bacterial quality, namely APC, total coliforms, E. coli and coagulase-positive

staphylococci. Only 24 hours are required for these rapid methods, resulting in a complete bacterial quality profile.

The most common method of preserving oysters is refrigeration at 32° - 35°F. The time and temperature of refrigerated storage greatly affects the shelf-life of oysters, as reported by numerous investigators (Tonney and White, 1926; Hunter and Harrison, 1928; Liebman et al., 1957, Kelly, 1960; Presnell and Kelly, 1961 and Hoff et al., 1967). Thus, it is essential to maintain proper storage conditions throughout harvesting, processing and distribution of oysters as well as in retail outlets.

In processing plants, oysters are stored at -20°C (National Shellfish Sanitation Program, 1965). It has been reported that storage below freezing temperature decreases bacterial growth (Tressler, 1937; Burton, 1949; Tennant, 1955; Ingraham, 1958 and Kelly et al., 1961). Kelly et al. (1961) stated that storage at -21°C greatly decreased bacterial growth. Varying storage times and temperatures were used in this investigation to determine to what extent the bacterial quality of oysters is affected.

It is generally agreed that numbers of bacteria in oysters vary according to harvest month. (Berry, 1916; Fisher and Acker, 1935; Lord, 1941; Wise et al., 1948; Kelly, 1960; Kelly et al., 1961; Presnell and Kelly, 1961; Hoff et al., 1967, Vanderzant et al., 1973 and Fugate, 1975). Oysters used in this investigation were obtained for a

period of 8 months, September, 1975 through April, 1976. Data obtained in this study demonstrate the effect of harvest month on bacterial numbers in oysters.

Many consumers prefer raw, unwashed (salty) oysters. The higher numbers of bacteria in these unwashed oysters decrease shelf-life and concomitantly increase the probability of illness. Because of this consumer custom, another aspect of this study was the determination of the bacterial quality of both washed and unwashed oysters.

Thus, there were several objectives of the work described in this dissertation. The primary objective was to determine the reliability of 2 different bacteriological procedures, rapid and official, for estimating the numbers of organisms conventionally classified as APC, total coliform, E. coli and coagulase-positive staphylococci in oysters. The effects of the following major sources of variation on the bacterial quality of oysters were also determined: (1) two washing treatments which yielded washed and unwashed (salty) oysters, (2) two levels of storage temperature, 0^0 and -20^0C, (3) three levels of elapsed storage time, 0 (receipt of oysters), 7 and 14 days for samples stored at 0^0C, and 0, 10 and 20 days for samples stored at -20^0C, and (4) eight monthly collections, September, 1975 - April, 1976.

REVIEW OF LITERATURE

I. Pollution of Oysters

Oysters, filter feeders, obtain their food from the waters overlying their beds (Truitt, 1929). Miller (1937) reported that between 25 and 50 gallons of water/day/oyster are filtered. These shellfish can secure sufficient food (algae, diatoms, etc.) under normal conditions to sustain life, grow and reproduce. Ingested food is digested in their stomach while non-digested materials are egested out through the intestines and anus. Consequently, if an oyster is harvested when its stomach or intestines contain matter inimical to humans and is then eaten raw or only partially cooked, the consumer may suffer ill effects. Outside the body of the oyster itself, but within the shell, there exists a small quantity of "liquor" which similarly may be a carrier of pathogenic organisms.

Within the oyster shell, there is a mucous secretion which clings to the oyster meat. Round (1916) suggested that this substance serves two purposes, first as a protective layer to the body of the oyster and second as a layer for the entrapment of food for the oyster. A large percentage of the bacteria in the water passing through the oyster is entrapped in this mucous.

From the public health viewpoint, it is unfortunate that those who most enjoy oysters seem to prefer them in the

raw state, since it is in this condition that they are most likely to cause illness. Hunter and Harrison (1928) suggested that even cooked oysters may be dangerous to consumers. The authors reported that no reliance can be placed on the ordinary methods of cooking, i.e., frying, scalloping and roasting, to render oysters which may contain pathogens safe for consumption.

The probability that sewage often contains infective agents has been recognized for many years. Because of the serious danger to public health, sewage pollution is a problem which has continually confronted oyster growers and public health officials.

The methods by which oysters from a given region are deemed polluted and the determination of the degree of pollution are similar to those methods used in the examination of water. Prescott et al. (1946), Kelly (1960) and Presnell and Kelly (1961), stated that special emphasis has been placed on the application of various tests for the determination of fecal organisms. In water, these bacteria are an index of pollution and their occurrence in appreciable numbers is considered an indication that shellfish may contain pathogenic organisms.

According to Hunter (1939), food cannot be placed in the same category as water when using the coliform group as an indicator of pollution. Although there are some foods which should not contain any coliforms, there are many foods which,

because of their nature or source, are subject to contamination by soil or non-fecal types of coliforms. Thus, Hunter recommended E. coli as the organism which should be used as an indicator of pollution.

Perry (1939) stipulated that the examination of shellfish and their growing waters cannot be considered in the same category with drinking water which can be filtered, chlorinated, etc. Perry attempted to correlate the relative incidences of E. coli and coliform organisms with probable pollution. He reported little difference in the number of total coliforms in water whether unpolluted, sightly polluted or highly polluted.

In another study, Perry and Bayliss (1936) found that other types of coliforms occurred less frequently and were of less importance since large numbers might be found in unpolluted waters. They suggested that E. coli is the logical bacterium to use as an index of fecal pollution since it is the most characteristic and constant coilform in the human intestines and in fresh human feces. E. coli has also been found in the intestinal tract of other warm-blooded animals, including animal pets (Galton et al., 1952), livestock (Nottingham and Urselmann, 1961), poultry (Quist, 1962) and wild animals (Lofton et al., 1962), this organism being acquired through contaminated food and water sources (summer, 1967). E. coli has been shown (Rottini and Zacchi, 1968 and APHA, 1970) to cause gatroenteritis characterized by a profuse watery diarrhea. In addition, Turck and Petersdorf (1962)

and Turck et al. (1969) reported that urinary infections are caused by E. coli as cited by Mitchell (1972).

Studies on the pollution of waters above oyster beds are conducted because they may indicate pollution of oysters in the area. Paponnet and Brisou (1937 a) found that 66% of oysters in one bed contained intestinal organisms. Later these authors (1937 b) found that 77% of the oysters in the same bed were contaminated by intestinal organisms. Fuller (1902) reported that oysters located one quarter of a mile away from a sewer opening contained E. coli and Aerobacter aerogenes (Enterobacter). Oysters from a bed 2 miles from the sewer contained these same organisms. He also reported that 30% of oysters in a bed situated in a strong tidal current 5 miles below the sewer contained E. coli. When oysters from a bed located 6½ miles below the sewer were bacteriologically examined, no E. coli was found.

Changing local conditions such as tide, temperature, etc. prohibit close agreement in results from examination of oysters and their overlying waters. Wise et al. (1948) reported that the coliform content of oysters increased as the temperature of the water decreased. Fisher and Acker (1935) reported that water and oysters showed lower counts of coliforms in the winter than in the summer. This latter seasonal variation in coliform counts of oysters has also been reported by several investigators (Gorham and Tonney, 1912; Round, 1916; White 1926; Lord, 1941; Arcisz, 1951; Wilson and

McCleskey, 1951; Kelly, 1960; Kelly et al., 1961; Presnell and Kelly, 1961; Hoff et al., 1967; and Cobelli and Hefferman, 1975.) In addition, Berry, (1916) and Vanderzant et al., (1973) and Fugate et al., (1975) reported that total bacterial counts were higher in summer months than in winter months.

Oysters can also be polluted during preparation for market. Old (1932) reported that the major sources of contamination are: (1) floating in polluted areas, (2) insanitary shucking and packing plants, (3) washing with polluted water or using contaminated ice and (4) unclean methods of handling, packing and shipping.

The possibility of polluting oysters by means of the fingers during handling was demonstrated by Tanon and Neveu (1932). These authors stated that bacterial contamination of oysters occurred when improperly stored or washed with contaminated water. Kelly (1961) and Presnell and Kelly (1961) suggested that significant increases in coliforms and plate counts occurred not only during harvesting, but also during handling and shipment. The knowledge of conditions under which shucking is carried out in many sections of the country indicates that pollution may occur through improper hygenic practices among workers, improper washing of the polluted material of the oyster and the use of polluted water in the ice. Several investigators, including Pottinger (1944) and Kelly (1961), and the U. S. Dept. of Health, Education and Welfare (1965) have described proper procedures for the processing of oysters.

II. Processing of Oysters

In 1658 the Dutch council of New Amsterdam enacted an ordinance governing the harvesting of oysters from the East River. The establishment of regulations for the harvesting and processing of oysters ensued. In 1925, at a conference sponsored by the U. S. Public Health Service, the following basic concepts of shellfish sanitation were recgonized: (1) shellfish are a valuable natural resource; (2) the cultivation, harvesting and marketing of shellfish are valuable components in the economic structure of many coastal states; (3) state and federal programs are essential for the safe use of shellfish and (4) transmission of disease is not to be tolerated. Also in 1925, cooperative control procedures were developed, including those for the state, the Public Health Service and the industry.

The present accepted procedures for the sanitation of shellfish were established in 1965 by the National Shellfish Sanitation Program (U. S. Dept. of Health, Education and Welfare, 1965). The following definitions were approved by the National Shellfish Sanitation Program and are presently used: (1) coliform group - all the aerobic and facultative anaerobic Gram-negative, non-spore-forming bacilli which ferment lactose with gas formation within 48 hours at 35°C. Those bacteria which produce gas from EC broth within 24 hours at 44.5°C in a water bath are considered fecal coliforms; (2) Most Probable Number (MPN) - a statistical estimate of the number of bacteria per unit volume, determined from the number of

positive results in a series of fermentation tubes and (3) satisfactory bacteriological standards for shucked oysters at the retail level - fecal coliform density not to exceed 230 MPN per 100 grams; total aerobic plate count not to exceed 500,000 per gram at 35°C

The National Shellfish Sanitation Program (U. S. Dept. of HEW, 1965) approved processing procedures for oysters including those for shucking, shell disposal, packing and refrigeration. Oysters shall be reasonably clean at the time of shucking so that large quantities of adhering material will not be mixed into the shucked stock during the shucking process, thus contributing to high bacterial counts in the final product. Oysters shall be shucked so that they are not subjected to contamination. Water used in fluming or washing shell stock shall be from an approved source of the state regulatory agency.

The bacterial count of the final pack is related to the time elapsed from shucking to attainment of a temperature of approximately 45°F, i.e., the period of time the oysters are held at a temperature favorable to the rapid growth of bacteria. Those factors in the shucking process which determine the length of time that shucked oysters are above 45°F include: (1) the speed of the individual shucker, (2) the quality and species of the oyster, (3) the air temperature, (4) the temperature of the oyster, and (5) the period of time for shucking containers to be delivered to the packing room. The total length of time that oysters may be held on the

shucking bench without causing high bacterial counts is dependent upon the size of containers, the temperature of the oysters, the method of cooling and the temperature of the blower water. The total elapsed time between shucking and the attainment of a temperature of 45°F should not exceed 4 hours.

The storage of shucked oysters on shucking benches for extended periods of time increases the possibility of contamination. Bacteriological examinations of water in dip buckets have shown very high coliform counts. Because these organisms may be carried into the shucked oysters the sanitary control of the water is essential.

Those shells from which meats have been removed shall be discharged promptly from the shucking room. Several methods of shell removal approved by the National Shellfish Sanitation Program (U. S. Dept. of HEW, 1965) include, but are not limited to, baskets, wheelbarrels, shell-drop-holes, conveyors or barrels.

Shucked oysters shall be packed without exposure to contamination. Clean, single service containers made of impervious materials or clean, returnable containers which are sealed so that tampering can be detected are permissible for the packing of shellfish. Packing oysters in unclean containers negates all precautions taken to produce a clean and safe product.

According to the National Shellfish Sanitation Program (U. S. Dept. of HEW, 1965), shucked oysters shall be cooled to an internal temperature of 45°F within 5 hours after shucking. Storage temperatures between 34° - 40°F is strongly recommended, with storage temperatures not to exceed 45°F. Temperatures for frozen storage rooms shall not exceed 0°F. Because oysters are an excellent medium for bacterial growth, packaged oysters should be cooled promptly. Moreover, temperatures exceeding 45°F may accelerate physical deterioration and spoilage of the shucked oysters.

III. Effect of Storage Conditions on the Bacterial Quality of Oysters

Two important factors in considering the storage of fresh shucked oysters are the speed of cooling after shucking and the storage temperature. Liebman et al. (1957) suggested that if these factors are not considered, it is possible that oysters can become undesirable and even dangerous. Because oysters ingest and concentrate bacteria present in the water from which they are taken, live in shallow coastal waters and are handled during preparation for market, various bacteria are often found in the edible portions. Thus, without proper preparation and storage, these bacteria can multiply rapidly and cause spoilage.

A study conducted by Kelly et al. (1961) in conjunction with the Washington State Department of Health and the Shellfish Sanitation Laboratory revealed that progressive but slight increases in coliform MPN's and plate counts were noted

during the shucking and packing operations. In contrast, little if any changes in fecal coliform MPN's were noted. These authors also reported that sudden increases in plate count are indicative of improper handling or refrigeration at the processing or marketing levels and the beginning of spoilage. While most of the microbial flora of oysters are not harmful to the consumer, small numbers of fecal organisms are sometimes found.

The optimal temperature for the growth of oysters is probably between 20°C and 25°C, according to Round (1916). He suggested that cells of oysters are most active in this temperature range causing mucus cells to secrete larger quantities of mucus and gills to filter larger quantities of waters than at lower temperatures. The authors stated that the more mucus secreted by the oysters, the greater the amount that will remain clinging to their body and the greater number of bacteria will be found in the mucus.

Round (1916) further suggested that as the temperature of the water decreased, the metabolic processes of oysters are correspondingly retarded causing a smaller quantity of water to be filtered. He thus reasoned that fewer bacteria will be found on the body of the oyster. Several other studies have shown that this seasonal variation in bacterial numbers in oysters exists (Berry, 1916; Wilson and McCleskey, 1951; Kelly, 1960; Presnell and Kelly, 1961; Kelly et al., 1961; Hoff et al., 1967; Vanderzant et al., 1973 and Fugate et al., 1975).

Several studies have been conducted on the effect of storage on changes in various bacterial indices in shucked oysters (Tonney and White, 1926; Hunter and Harrison, 1928 and Wilson and McCleskey, 1951). These investigations involved oysters of only a few lots, not freshly harvested in all cases and taken at irregular intervals as reported by Hoff et al. (1967). Therefore, Hoff et al. (1967) conducted studies of oysters based on a large number of lots collected at the point of harvest, stored under carefully controlled conditions and examined at regular intervals. They reported that at a storage temperature of 10°C, increases in total bacterial counts, coliform MPNs and fecal coliform MPNs were greater than at 3°C and iced.

In a study by Liebman et al. (1957) it was found that storage temperature is an important factor with respect to the rate of spoilage of freshly shucked oysters. Rapid coolings and lower temperatures, i.e., 32°F, will prolong the storage life, while slow cooling and high storage temperatures will be conducive to rapid spoilage and a short shelf-life.

Oysters are commonly stored at temperatures a little above freezing. Wilson and McCleskey (1951) stated that although pathogenic intestinal bacteria are unable to multiply at this temperature, coliform organisms may be able to increase. Hunter and Harrison (1928) reported that E. coli and other lactose fermenting bacteria found in oysters are unable

to multiply when the storage temperature is below 10°C. Parsons, according to Hunter and Harrison (1928), also stated that E. coli would not multiply in oysters as to affect the bacterial score. On the other hand, Tonney and White (1926) and Murchelano et al. (1975) reported that E. coli and other organisms would multiply in shucked oysters stored at 5°C to 20°C. However, Tressler (1937), Burton (1949), Kelly et al. (1961), Ingraham (1958) and Tennant (1955) reported that lower temperatures caused decreases in bacterial numbers.

The best initial means of preventing bacterial spoilage of oysters and the associated dangers are the minimizing of contamination of clean growing areas and the maintenance of careful sanitation during processing. Proper storage conditions are necessary for keeping bacterial growth at a minimum level until the oysters reach the consumer.

IV. Bacteriological Procedures for the Examination of Oysters

Development of the "Official Methods"

Upon the discovery that diseases might be transmitted to man by consuming sewage polluted shellfish, the American Public Health Association (APHA) became involved in the establishment of a standard procedure for examination. The APHA catalogues the methods already being used.

The first method to be devised and published was presented by Klein (1896). Each oyster was carefully washed and brushed in a small quantity of sterile water, dried with a clean cloth, opened with a sterile knife and its body mashed

up with the liquor contained in its shell. Both classes of material, that obtained from the outside of the oyster and that (including water) which had been contained within its shells, were now ready for detailed examination. Portions of the sterile water used for cleansing were transferred to phenol broth and phenolated gelatin plates which were incubated. If, at the end of 24 hours, indications of microbial growth appeared in the broth, further phenolated gelatin plates were inoculated therewith. As soon as colonies appeared on these plates, they were carefully examined under a magnifying glass and those colonies which resembled E. coli or the typhoid bacillus were subjected to microscopic tests. Klein later modified his own procedure by omitting the examination of the washings of the shell and specifying the amount of juice and shell liquor to be used for inoculation purposes.

Houston (1904) devised a method for the examination of oysters for the indication of fecal pollution that was supeior to Klein's because it was strictly quantitative. For each examination, the volume of oyster plus liquor was measured so that oysters, being of medium size and containing a medium amount of liquor, 100 cc., might be considered a fair average. Results of Houston's examinations were expressed as numbers of bacteria, either per oyster or per ml. of oyster.

Criticizing Houston's method as being too cumbersome, Johnstone (1909) devised a simplified method for routine use

in the examination of oysters. This was accomplished by using a lactose medium suggested by Grunbaum and Hume (1902) for the detection of fecal pollution.

At the beginning of the twentieth century, interest in the testing of shellfish for fecal pollution was greatly increased. Eyre (1924) cited the work of Gage (1910) who presented a radically new procedure which involved the grading of oysters into more than two categories, i.e., "passed" or "rejected". Gage suggested that a positive test in 50% or more of the samples indicated fecal pollution of a more or less dangerous character and further suggested that a negative test in 80% of the samples indicated reasonable safety.

Stiles (1911) made bacteriological examinations of oysters from the Atlantic and Gulf Coasts. In his studies, Stiles suggested specific procedures for the collection and preparation of samples and also developed a very elaborate procedure for the plating of organisms.

The need for developing a standard method from such a state of chaos was first recognized in 1905 by the APHA. Hammer (1948) states that in that year a committee was appointed to recommend such a method. A standard methods manual was finally published outlining the procedure for the aerobic plate count (APC) in 1910 (APHA, 1910).

The APC is the most widely used microbiological test and according to one investigation, Kachikian et al. (1959)

the most valuable. As cited by Weinziel and Newton (1914), the first report of an aerobic plate count of meats was that of St. John in the late nineteenth century. Eyre (1913) recognized a problem of uniformity in the technique and developed a method for the APC. However, the method was not widely accepted as a standard procedure and the problem of uniform methodology continued. In fact, Weinziel and Newton (1914) reported that workers began developing procedures without regard for uniformity. Geer et al. (1932) reported that as late as 1932 no standard for the bacteriological examination of meats was accepted.

Hunter (1934) expressed his concern for uniform methodology while investigating the processing of crustacea. He suggested that one method of analysis should be acceptable to all agencies which have a role in the sanitary control of foods.

The aerobic plate count, though now accepted as a criterion for quality of seafood, was frequently tested and criticized. The reproducibility of the aerobic plate count procedure was assessed by Schacht and Robertson (1932) who reported great differences among individuals. The major causes of error in plate counts were failure to know what to count, failure to detect colonies and carelessness. Davis (1969 a) reported that the reproducibility of the APC decreased with an increase in the number of bacteria in the sample being tested. The author reported close agreement

among different laboratories when low count samples were examined for APC but not when high count samples were used. Davis (1969 b) also reported that a competing flora may produce variation in the APC. Davis (1971) suggested that other factors affecting the reproducibility of the APC were immediate treatment, method of transport, holding of the sample in the laboratory and all aspects of the methods used, i.e., quantity used in enrichment, media and temperature of incubation.

Additional factors affecting the accuracy of the aerobic plate count have been reported. Multiplication during diluting and plating was reported by Mudge and Lawler (1928) to produce variation in the procedure and clumping of bacteria was reported by Jennison (1937) to be a major factor. Sampling error and dilution error were reported by Jennison and Wadsworth (1940) to be additional factors.

When the APC is applied to seafood products, other variables exist. In their investigation using the pour plate method, ZoBell and Conn (1940) found that melted agar and warm temperatures decreased microbial recovery. This study was supported by the findings of Klein and Shenyuk (1974). The investigators demonstrated a highly significant difference between results of pour plate and spread plate techniques and suggested that the temperature of the molten agar caused a decrease in the numbers of bacteria.

Recognition of the limitations of the aerobic plate count have been suggested by most investigators. Jennison and Wadsworth (1940) stated that relative rather than absolute numbers of cells must be reported for the APC to be comparable. Corlett (1974) suggested that if the APC was used correctly, it could be used to determine the effects of harvesting, storage, processing, preservation and packaging. Despite reports of its questionable accuracy, the APC is presently one of the primary tests of the quality of foods.

There are several official procedures for the aerobic plate count. Although the titles of the procedures vary, i.e., "official procedures" (AOAC,1970), "standard methods" (APHA,1965, 1967), "recommended methods" (APHA,1966) or "recommended procedures" (APHA,1962,1970), they are basically identical. The solid food is homogenized in phosphate-buffered diluent similar to that described by Butterfield (1932), diluted in the same diluent, plated out in decimal aliquots in Plate Count agar and incubated at 32^0C (APHA, 1965), 35^0C or 20^0C (APHA,1967) or 35^0C (AOAC, 1970) for 48 hours.

Other bacteriological quality tests used in foods are coliforms, including E. coli. The work of Von Fritsch in 1880 on the potability assessment of water was cited by Clark and Kabler (1964) as the first reported use of E. coli as a possible quality criterion. Escherich (1885) suggested that two organisms, later classified in the genera Escherichia and

Aerobacter (Enterobacter), when found in water constitute a hazard since fecal pollution may be implicated. It was assumed that the presence of these two organisms indicated an increase in the probability of enteric pathogens introduced into water by infected persons.

Eijkman (1904) suggested separation of fecal and non-fecal coliforms by incubation at elevated temperatures. This technique was not immediately accepted. Parr and Kaufmann (1951) stated that a single laboratory procedure could not differentiate between coliforms of fecal and non-fecal origin. Parr (1939) further suggested that additional tests be performed, including indole, methyl red, Voges-Proskauer and citrate utilization. It has been shown that these additional tests are not required in routine quality assessment tests. In their investigation, Hajna and Perry (1935) reported that Eijkman's elevated temperature tests for fecal coliforms could be modified by using a special medium.

E. coli has been accepted as the valid coliform of fecal origin and thus, of public health significance. Griffin amd Stuart (1940) greatly influenced this acceptance by their environmental investigation wherein they isolated over 6000 coliforms from milk, water, soil, drains and feces. The investigators consistently isolated E. coli from feces but only rarely from other environments. Also, the other coliforms were rarely isolated from the feces.

Non-fecal coliforms have been accepted as suitable indices of poor processing procedures and sanitation habits in

many food products. Sherman and Wing (1933) and Thomas (1955) have suggested that the test for non-fecal coliforms is a valuable index of proper processing of milk. Moreover, it has become a standard test in the processing and quality assessment of seafood.

Official procedures for the enumeration of total coliforms are outlined in the Bacteriological Analytical Manual for Foods (U. S. Dept. of Health, Education, and Welfare; 1969), Association of Official Analytical Chemists (AOAC, 1970) and Recommended Procedures for the Examination of Sea Water and Shellfish (APHA, 1970). This official procedure is basically a MPN technique introduced and improved by Hopkins (1933, 1934). Two separate MPN procedures employing two different broths are used. Four days from the time of homogenization of the sample, the results are obtained.

Official techniques for enumeration of "fecal coliforms" or E. coli are also MPN procedures. Official procedures are presented along with that of the total coliforms (AOAC, 1970; U. S. Dept. of Health, Education and Welfare, 1969; and APHA, 1970).

Coagulase-positive staphylococci are considered indicator organisms in the seafood industry. In 1884, Rosenback described the type species for the genus Staphylococcus (Breed et al., 1957). Following the occurrence of numerous cases of severe illnesses attributed to the consumption of cheddar cheese, Vaughn (1884) microscopically examined

samples of the cheese and concluded that spherical bacteria produced a chemical in the food that rendered it toxic. The first definite case of staphylococcal food intoxication occured in 1914 after consumption of milk. Staphylococci were consistently isolated from the incriminated cows.

Jordan (1931) reviewed several outbreaks of staphylococcal food intoxications. He cited Dack et al. (1930) who isolated the etiological agent from cake. He also cited Dolmann and Wilson (1938) who reported that symptoms of the staphylococcal intoxications were reproduced when sterile filters of broth used to culture the organisms were administered to human volunteers. Staphylococcal food poisoning has been reported as the most common type of food intoxication. Olsen (1968) suggested that several million cases of Staphylococcus food poisoning occur annually.

Several indices of enterotoxigenicity for staphylococci have been reported. Chapman (1944) and Evans and Niven (1950) suggested that production of coagulase was an indication of enterotoxigenicity. Bergdoll et al. (1967) and Thatcher and Simmons (1956) reported, however, that coagulase-negative staphylococci also produced enterotoxin. Feldman (1946) suggested production of hemolysin as an index of enterotoxin production. Several investigators (Gwatkin, 1937; Joshi and Dale, 1963 and Zemelman and Longeri, 1965) have reported the fermentation of mannitol as an indicator of enterotoxigenicity.

Minor and Marth (1971) suggested that staphylococci possesses no single physiological characteristic that is an absolutely reliable index of enterotoxigenicity. It is agreed generally, however, that coagulase-positivity is the most suitable criterion.

The presently accepted official procedure for the enumeration of coagulase-positive Staphylococcus is a 3 X 3 MPN. Media employed in the procedure are Trypticase Soy broth, Vogel-Johnson agar, nutrient agar and Brain Heart Infusion broth.

Official methods, when performed properly, are used to assess the quality of food and thus protect consumers. However, they are very time consuming and thus would delay distribution of oysters. Rapid, reliable bacteriological tests are definitely needed in the oyster industry.

Development of "Rapid Methods"

As early as 1912, rapid procedures were used for bacteriological examinations of foods. Frost (1912, 1921) suggested a method which was reported to be comparable to those of the standard aerobic plate count procedures for the examination of dairy products (Hatfield and Park, 1922). He inoculated agar drops, allowed them to solidify on microscope slides, incubated them for 4 hours and then counted resultant colonies under low power. His method became known as the "little plate method". Winter et al. (1971) used a method involving membrane filters. The author stated that by

rinsing microbial cells from foods and concentrating the diluent on the surface of the membrane filter quantitatively, a total bacterial count could be performed in approximately 6 hours. The seeded membrane filters were incubated on absorbent pads saturated with nutrient broth, fixed in janis green and the resulting microcolonies counted under low power.

The most recent rapid method developed from the aerobic plate count was that of Sharpe et al. (1972) and Sharpe and Kilsby (1972) who described their procedures as "agar droplets". A 0.1 ml sample suspension in molten agar was placed in petri plates, incubated at 35^0C and resultant microcolonies counted after 8 hours. After the modification of the droplet procedure (Sharpe et al., 1972), the authors stated that the method would be suited for quality control in the food industry.

Quantitative recovery of coliforms and E. coli from foods using rapid methods have been reported by several investigators. Methods involving the plugging of fermentation tubes with stoppers have been suggested. Davis (1939) suggested that when stoppers were used, the gas produced from the fermentable carbohydrate was more readily noted. Using semi-solid media, Bicknell et al. (1952) stated that entrapped gas could be observed more rapidly than the conventional Durham tube. An agar overlay method was used by Moldovan (1935, 1965) who stated that all of the gas could be entrapped instead of only that produced at or below the Durham tube opening. Moreover, Moldovan reported that the overlay

method permitted a greater and more rapid recovery of coliforms. McDade and Weaver (1959) suggested that the agar overlay was more accurate than the conventional Durham tube whereas Stuart and Weaver (1948) suggested that it was at least as accurate as the Durham tube. Kendall (1973) used an agar-plugged inverted tube technique and massive inocula to determine coliform and E. coli MPN results. This rapid method required less than 12 hours.

Simplicity and relative low cost have made the Durham tube procedure the most widely used despite numerous suggested rapid methods. A rapid method using a Durham tube MPN procedure was described by Fishbein et al. (1967) for the recovery of E. coli. The investigators performed a 3 X 3 MPN in LST at 44.5°C for 24 hours. Fishbein (1962) reported that LST promoted greater cell recovery than the official EC broth.

Very few studies have been conducted on the detection of coagulase-positive Staphylococcus aureus. The need for more rapid methods for this organism was suggested by Minor and Marth (1971) in their review of present methods of enumeration.

A rapid method for the detection of coagulase-positive organisms was described by Wilson et al. (1959). A suspension of homogenized sample was transferred to a BHI medium containing mannitol and salt and incubated at 35°C with shaking for 4 to 6 hours. The broth was examined for Gram-

positive organisms and a coagulase test performed on typical isolates. Chesbro and Auborn (1967) devised a rapid method that required only 3 hours. The authors reported that Staphylococcus aureus produced a stable deoxyribonuclease. The nuclease was extracted from the food sample and measured quantitatively. The method was based on the assumption that only enterotoxigenic staphylococci produced the enzyme. Lachica et al. (1971) reported that 95% of the enterotoxin producing strains studied produced the nuclease and 93% of those strains were coagulase-positive. Brandish and Willis (1970) and Lachica et al. (1969) reported similar results.

The present accepted medium for the aerobic plate count is Plate Count agar. However, Nickerson and Sinskey (1972) reported that Eugon agar yields higher counts with seafoods. This was attributed to the greater availability of dextrose and the resultant increase in metabolic rate of the organisms. The accepted 48-hour incubation period is recommended for routine plating procedures. Longer incubation times have been suggested for the enumeration of psychrophiles (Nelson and Barder, 1954; Vanderzant and Moore, 1955).

A MPN procedure for the enumeration of coliforms and E. coli in foods is the accepted method. However, an official MPN method for the enumeration of "fecal coliforms" in foods has been presented (U. S. Dept. of Health, Education and Welfare, 1969) which requires only 24 hours incubation

time. Basically, it is an elevated temperature method similar to that described by Fishbein et al. (1967). A total of 48 hours is required for the entire procedure because plating on differential agar is needed.

The present accepted method for the enumeration of coagulase-positive Staphylococcus aureus requires 5 days. However, there are at present two official methods which require less time. BAM (U. S. Dept. of Health, Education and Welfare, 1969) described a direct plating method using a medium introduced by Baird-Parker (1962). The method is a spread technique whereby typical colonies are counted and tested for coagulase production. The second method requires 4 days and is basically a 3 X 3 MPN procedure in selective broth and differential agar. Typical colonies are tested for the production of coagulase.

A method recommended by the Food Research Institute (University of Wisconsin) was described by Foster et al. (1970). The procedure is similar to the official direct plating method. Other direct plating methods were reported by Thatcher and Clark (1968). The authors suggested five procedures using Baird-Parker, Egg Yolk-Azide, Milk Salt, or Tellurite-Polymixin-Egg Yolk agars and one procedure requiring enrichment in a selective broth followed by plating on differential agar.

EXPERIMENTAL PROCEDURE

I. Methods

Oyster Samples

The oysters used in this study were the Crassostrea virginica species, collected monthly from beds in Gulf Coast waters near New Orleans over an eight-month period which began in September, 1975. Each monthly collection was transported from the oyster beds in a refrigerated truck at 40°F to New Orleans where the oysters were shucked upon arrival by professional shuckers using facilities which conformed to FDA regulations (FDA Definitions and Standards for Shellfish, Title 21, Part 36, Section 36.10). Each month, six one-pint samples of freshly shucked, unwashed oysters contained in glass jars were placed in a chest containing ice and then transported to the Food Science Department at Louisiana State University in Baton Rouge. No more than two days elapsed from the time the oysters were removed from the water until the laboratory tests were initiated.

Upon arrival at the laboratory, the six one-pint samples of oysters were divided into two equal lots. Lot A, consisting of Jars I, II and III, was used for studies on unwashed (salty) oysters, and Lot A', consisting of Jars I', II' and III', was used for similar studies on washed oysters. Aliquots of unwashed oysters were removed from the jars and

microbiological analyses at various times during cold storage according to the scheme shown in Figure 1; the same scheme was used for removing aliquots of washed oysters for analysis.

Lot A: Unwashed Oysters

From Jar I a 200-gram aliquot of unwashed oysters was removed and diluted 10^{-1} with Butterfield's phosphate buffer. Homogenization was accomplished over a 2-minute period in a sterile stainless steel Waring Blender. Bacteriological analysis of the homogenate was initiated immediately to yield counts of organisms in unwashed oysters as received (day 0). The oysters remaining in Jar I were then stored at 0°C for 7 days before undergoing bacteriological analysis.

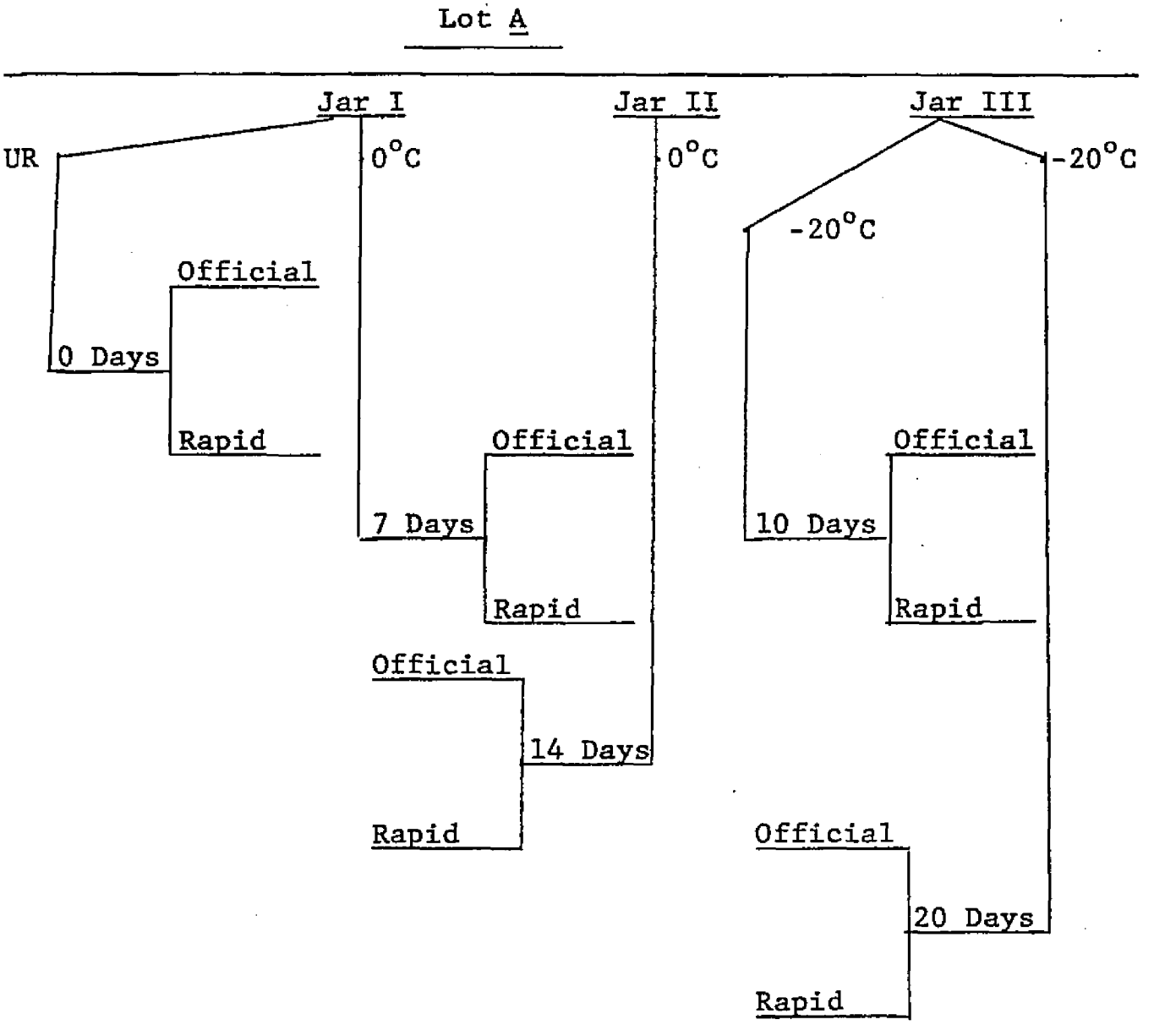
The pint sample of unwashed oysters in Jar II was stored at 0°C for 14 days before being analyzed.

The pint sample of unwashed oysters in Jar III were divided into two sterile containers. Both containers were then stored in a freezer at -20°C. Bacteriological tests were performed after 10 and 20 days.

Thus, the 3 one-pint samples of oysters comprising Lot A, were used to determine the bacterial quality of unwashed oysters upon receipt and at various times during iced and frozen storages; 200-Gram aliquots were used for all tests.

Lot A': Washed Oysters

The remaining one-pint samples of unwashed oysters (designated as Lot A') were immediately washed in running tap water for two minutes and allowed to drain for a period of



UR = Upon Receipt

Fig. 1- Scheme Used for the Removal of Aliquots of Stored Oysters for Bacteriological Analyses

five minutes on a stainless steel strainer having 0.25" diameter perforations located not more than 1.25" apart. The oysters were distributed evenly over the surface of the strainer but were not otherwise agitated during the draining period. The washed and drained oysters which comprised Lot A' (Jars I', II', III'), were bacteriologically examined using the same storage conditions as those described above for Lot A.

Thus, the 3 one-pint samples of oysters comprising Lot A' were used to determine the bacterial quality of washed oysters upon receipt and at various times during iced and frozen storages; 200-Gram aliquots were used for all tests.

Methods of Analysis: Official and Rapid Methodology

Two methods were used for the enumeration of APC organisms, total coliforms, E. coli and coagulase-positive staphylococci in oysters. The standard reference was those official procedures in Official Methods of the Association of Official Analytical Chemists (AOAC, 1970). The second method was a series of rapid procedures. Those rapid procedures for the enumeration of APC, total coliforms and coagulase-positive staphylococci were developed by investigators of the LSU Department of Food Science and require only 24 hours for completion. The rapid procedure for the enumeration of E. coli was described by Fishbein et al. (1967) and also requires only 24 hours for completion.

Aerobic Plate Count

Official: Plate Count agar was used in the procedure. Dilutions were prepared using Butterfield's buffer. The

dilutions used were 10^{-1} to 10^{-6} in duplicate. The sterile agar was held at 45° - 47°C and poured at that temperature. The agar was allowed to solidify, inoculated, inverted and incubated for 48 hours at 35°C. The resulting colonies were counted on a Quebec colony counter using standard counting procedure.

Rapid Method: Eugon agar was used in this procedure. Dilutions were prepared in Butterfield's buffer. The dilutions used were 10^{-1} to 10^{-6} in duplicate. The sterile agar was held at 45° to 47°C and poured at that temperature. The plates were allowed to solidify, inoculated, inverted and incubated at 35°C for 24 hours. The resulting colonies were counted on a Quebec colony counter using standard counting procedure.

Total Coliforms

Official: A 3 X 3 MPN using Lauryl Sulfate Tryptose Broth (LST) and dilutions of 0.1, 0.01 and 0.001 was incubated at 35°C for 48 hours. Gassing tubes were then transferred to Brillian Green Lactose Bile Broth 2% (BGLB) using a 3mm loop. The BGLB tubes were set up to retain the 3 X 3 MPN pattern. These tubes were incubated at 35°C for another 48 hours. MPN codes were taken from the positive BGLB tubes afer the incubation period and their numbers recorded.

Rapid Method: a 3 x 3 MPN using LST broth and dilutions of 0.1, 0.01 and 0.001 was incubated at 35°C for 24 hours. MPN codes were taken from the gassing tubes after the

incubation period and their numbers recorded.

Escherichia coli

Official: Gassing tubes from the LST MPN of the official coliform procedure were used to inoculate EC broth to retain the 3 X 3 MPN pattern. The EC broth was incubated at 44.5°C for 48 hours. Growth from positive tubes were used to streak Eosin Methylene Blue agar (EMB). Typical colonies were noted and a MPN code taken from the plates after an incubation period of 24 hours at 35°C.

Rapid Method: A 3 X 3 MPN using LST broth and dilutions of 0.1, 0.01 and 0.001 was incubated in a 44.5°C water bath for 24 hours. MPN codes were taken from the positive tubes after the incubation period.

Coagulase-Positive Staphylococci

Official: A 3 X 3 MPN using Trypicase Soy broth and dilutions of 0.1, 0.01 and 0.001 was incubated at 37°C in a water bath for 48 hours. After the incubation period, positive tubes were transferred to Vogel-Johnson agar and incubated for 24 hours at 35°C. Typical colonies were picked from the plates and streaked onto nutrient agar slants which were incubated for 18 hours. This growth was used to inoculate 0.2 ml of Brain Heart Infusion (BHI) broth with incubation for another 18 hours. After the incubation period, 0.5 ml of rehydrated coagulase plasma EDTA was added to the tubes and incubated for 4 hours at 37°C. Tubes showing

coagulated plasma were recorded and used to determine the final MPN of coagulase-positive staphylococci.

Rapid Method: A 3 X 3 MPN using Tryptic Mannitol Meat broth (TMM) 0.1, 0.01 and 0.001 was incubated for 24 hours in a 37°C water bath. Tubes indicating an acid reaction by the phenol red indicator were considered positive and used to establish the code for the MPN table listed in the AOAC manual (AOAC, 1970).

II. Materials

Media and Reagents

The composition of all media used in this investigation is listed in Tables A through J in the Appendix. All media were sterilized by autoclaving at 121°C for 15 minutes.

Butterfield's Phosphate Buffer

All dilutions were made with Butterfield's phosphate buffer (Butterfield, 1932). A stock solution was prepared by dissolving 24 grams of potassium acid phosphate (KH_2PO_4) in 500 ml of distilled water. The pH was adjusted to 7.2 with 1N NaOH and the mixture diluted to one liter with distilled water. The stock solution was stored at 4°C until needed. Diluents were prepared by adding 1.25 ml of the stock solution to one liter of distilled water and adjusting the pH to 7.2 with 0.1N NaOH. The buffer was sterilized by autoclaving at 121°C for 15 minutes.

Aerobic Plate Count

Plate Count Agar is the standard medium for the enumeration of bacteria in milk, water, waste water, and foods (APHA, 1965, 1966, 1967, 1970; AOAC, 1970). The medium was prepared by dissolving 24 grams in 1000 ml of distilled water.

Eugon agar has been reported to yield higher recovery of bacteria from seafoods (Nickerson and Sinskey, 1972), mainly attributed to the greater availability of dextrose which allowed more rapid colony production than standard Plate Count agar. Thus, Eugon agar was chosen as the medium for the rapid aerobic plate count technique. To rehydrate the medium 45.5 grams were dissolved in 1000 ml of distilled water (Difco, 1953).

Total Coliforms and Escherichia coli

LST is the standard medium for the presumptive determination of coliforms in water, waste water and foods (APHA 1965, 1966, 1967, 1970; AOAC, 1970). Tubes from the MPN procedures showing gas production were transferred from the LST to EC medium. The EC medium is recommended for the procedure in the determination of E. coli.

It has been reported that LST is less inhibitory than EC, thus yielding greater recovery of E. coli at elevated temperatures (Fishbein et al., 1967). LST was chosen as the MPN medium for the rapid precedure for E. coli, and was retained for the rapid test for total coliforms. Preparation of the medium was prepared by dissolving 35.6 grams of the

dehydrated medium in 1000 ml of distilled water. EC medium is recommended as a secondary confirmatory medium in the examination of water, waste water and foods for E. coli when used at 44.5 or 45.5°C (APHA, 1965, 1966, 1967, 1970; AOAC, 1970). EC medium was prepared by dissolving 37 grams of dehydrated powder in 1000 ml of distilled water (Difco, 1953).

BGLB 2% is the confirmatory medium for the determination of coliforms in water, waste water and foods (APHA, 1965, 1966, 1967, 1970; AOAC, 1970). Primary LST MPN tubes showing gas production after 48 hours are used as inocula in BGLB. To prepare the medium, 40 grams of dehydrated powder were dissolved in 1000 ml of distilled water (Difco, 1953).

Eosine Methylene Blue agar is recommended as a confirmatory differential plating medium for E. coli (APHA, 1965, 1966, 1967, 1970; AOAC, 1970). It is suggested that growth from the elevated temperature MPN positive tubes be streaked onto EMB and typical E. coli colonies counted. The medium is prepared by dissolving 37.5 grams of dehydrate into 1000 ml of distilled water and autoclaving (Difco, 1953).

Coagulase-Positive Staphylococci

Trypticase Soy broth is recommended as the MPN medium for enumeration of coagulase-positive staphylococci in the official procedure (AOAC, 1970). To increase the selectivity of the medium, 10% NaCl is added. To prepare the medium, 30 grams of the dehydrate are dissolved in 1000 ml of distilled water and dispensed in 9 ml amounts in screwcap tubes.

Vogel-Johnson agar is recommednded for isolation of coagulase-positive, mannitol fermenting staphylococci from foods (AOAC, 1970). The medium is used as a secondary MPN procedure in conjunction with Trypticase Soy broth. Typical colonies are produced by mannitol fermenting staphylococci capable of reducing potassium tellurite. To prepare the medium, 60 grams of dehydrate are dissolved in 1000 ml of distilled water and autoclaved (Difco, 1953).

Tryptic Mannitol Meat broth (TMM) was chosen for the 24-hour MPN of coagulase-positive staphylococci due to its ability to differentiate mannitol-positive staphylococci from negative strains. Several workers have reported that the fermentation of mannitol by Staphylococcus is a reliable indication of enterotoxigenicity and coagulase positivity, especially under low oxygen tension of anaerobiosis (Gwatkin, 1937; Joshi and Dale, 1963; Zemelman and Longeri, 1965). The TMM was dispensed into screwcap tubes and autoclaved, after which they were tightened while hot to maintain low oxygen concentrations. Phenol red was added to indicate acid production from the fermentation of mannitol with 10% NaCl and Polymyxin B as selective agents. The medium was prepared by dissolving 46 grams of the dehydrate in 180 ml of distilled water. Commercially available Polymyxin B solution was added to yield a final measure of 7,500 units per 9-ml tube. The medium was dispensed in the screwcap tubes and autoclaved.

BHI is a broth especially designed for cultivation of fastidious pathogenic bacteria. It is a recommended official procedure (AOAC, 1970) as the final culture medium for isolates prior to testing for coagulase production. To prepare the broth, 37 grams of dehydrate are dissolved in 1000 ml of distilled water (Difco, 1953).

Statistical Evaluations

Statistically, the data for iced oysters were analyzed separately from the data for frozen oysters. In both instances the duplicate measures obtained from 80 aliquots of oysters were subjected to an analysis of variance corresponding to a split plot design which evaluated the effects of (A) washing treatment, (B) laboratory methodology, (C) elapsed time during storage, and (D) month of harvesting, on the mean numbers in oysters of microbiological organisms as categorized according to four microbiological procedures designated as APC, coliforms, E. coli, and coagulase-positive staphylococci. For both iced and frozen oysters, washing treatment, elapsed time during storage, and month of harvesting, together with the interactions, treatment x months, elapsed time x months, treatment x elapsed time, and treatment x elapsed time x months, were regarded as main plots; laboratory methodology and the seven interactions involving this variable were regarded as subplots. All measurements were evaluated for statistical significance with the cooperation of the LSU System Network Computer Center, Department

of Experimental Statistics, using the Statistical Analysis System (SAS) developed by Barr and Goodnight (1971).

RESULTS AND DISCUSSION

I. Aerobic Plate Count Values of Oysters

In Tables I - IV are presented in chronological sequence for the eight months period, September, 1972 through April, 1976, the numbers of APC organisms found in oysters in duplicate determinations as estimated by each of the 2 microbiological procedures, rapid and official, which are being compared in these experiments.

In data columns 1 - 3 of these tables are given the values associated with the official method, and in columns 4 - 6 are the corresponding values associated with the rapid method. The data obtained for washed oysters are in Tables I and II and those for unwashed oysters are in Tables III and IV.

The individual values associated with the official method expressed as hundreds that were obtained for the 8 duplicate monthly groups of washed freshly harvested oysters (column 1) during the period, September, 1975 through April, 1976, ranged from 55 in January to 116 in April, with the overall mean for the 16 individual values being 87.6. The corresponding values associated with the rapid method (column 4) ranged from 56 in January to 144 in September, with the overall mean value being 78.1.

For the corresponding unwashed group of oysters (Tables III and IV), the official method gave values ranging

Table I Aerobic Plate Count Values of Washed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (APC/g x 100) Upon Receipt	7 Days	14 Days	Rapid Method (APC/g x 100) Upon Receipt	7 Days	14 Days
9/75	96.0	86.0	9200.0	106.0	84.0	10000.0
	102.0	85.0	9900.0	114.0	87.0	10500.0
10/75	92.0	91.0	8060.0	78.0	77.0	7600.0
	74.0	76.0	7300.0	78.0	73.0	7850.0
11/75	92.0	256.0	9000.0	79.0	198.0	9050.0
	80.0	176.0	6500.0	79.0	212.0	6800.0
12/75	84.0	218.0	7150.0	70.0	167.0	6400.0
	92.0	250.0	7700.0	72.0	171.0	6700.0
1/76	55.0	127.0	4900.0	56.0	136.0	5300.0
	60.0	131.0	5100.0	56.0	130.0	4950.0
2/76	84.0	185.0	8250.0	70.0	218.0	8250.0
	88.0	230.0	8300.0	73.0	235.0	8700.0
3/76	95.0	198.0	9100.0	85.0	176.0	8350.0
	90.0	180.0	9400.0	87.0	185.0	9400.0
4/76	116.0	99.0	9800.0	77.0	66.0	9990.0
	102.0	89.0	8950.0	70.0	61.0	8900.0

Table II Aerobic Plate Count Values of Washed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (APC/g x 100) Upon Receipt	10 Days	20 Days	Rapid Method (APC/g x 100) Upon Receipt	10 Days	20 Days
9/75	96.0	56.0	99.0	106.0	53.0	100.0
	102.0	54.0	110.0	114.0	53.0	99.0
10/75	92.0	43.0	85.0	78.0	41.0	67.0
	74.0	44.0	69.0	78.0	38.0	68.0
11/75	92.0	37.0	95.0	79.0	40.0	82.0
	80.0	29.0	75.0	79.0	33.0	83.0
12/75	84.0	33.0	68.0	70.0	37.0	66.0
	92.0	45.0	70.0	72.0	36.0	72.0
1/76	55.0	23.0	57.0	56.0	22.0	58.0
	60.0	22.0	59.0	56.0	21.0	49.0
2/76	84.0	39.0	86.0	70.0	41.0	72.0
	88.0	39.0	69.0	73.0	31.0	70.0
3/76	95.0	38.0	90.0	85.0	40.0	86.0
	90.0	36.0	91.0	87.0	38.0	88.0
4/76	116.0	52.0	94.0	77.0	39.0	74.0
	102.0	53.0	95.0	70.0	38.0	61.0

Table III Aerobic Plate Count Values of Unwashed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (APC/g x 100) Upon Receipt	7 Days	14 Days	Rapid Method (APC/g x 100) Upon Receipt	7 Days	14 Days
9/75	415.0	465.0	11500.0	400.0	420.0	10500.0
	415.0	435.0	10000.0	425.0	595.0	11000.0
10/75	268.0	315.0	9450.0	305.0	345.0	8950.0
	325.0	380.0	9700.0	410.0	410.0	11500.0
11/75	345.0	1090.0	19800.0	330.0	1020.0	20500.0
	335.0	1120.0	21000.0	310.0	1030.0	20000.0
12/75	315.0	1290.0	24200.0	310.0	1260.0	24000.0
	315.0	1340.0	22500.0	310.0	1220.0	20800.0
1/76	208.0	515.0	9800.0	223.0	665.0	9350.0
	195.0	390.0	7800.0	209.0	625.0	7500.0
2/76	256.0	630.0	9500.0	231.0	695.0	13800.0
	248.0	490.0	5400.0	216.0	845.0	16800.0
3/76	380.0	1595.0	27000.0	310.0	1585.0	36500.0
	375.0	2250.0	45000.0	300.0	1205.0	24000.0
4/76	325.0	480.0	9900.0	365.0	460.0	11000.0
	390.0	495.0	12500.0	425.0	510.0	11500.0

Table IV Aerobic Plate Count Values of Unwashed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (APC/g x 100) Upon Recepit	10 Days	20 Days	Rapid Method (APC/g x 100) Upon Receipt	10 Days	20 Days
9/75	415.0	239.0	480.0	400.0	224.0	395.0
	415.0	250.0	360.0	425.0	305.0	335.0
10/75	268.0	126.0	291.0	305.0	195.0	285.0
	325.0	164.0	465.0	410.0	203.0	450.0
11/75	345.0	174.0	310.0	330.0	163.0	305.0
	335.0	132.0	265.0	310.0	159.0	310.0
12/75	315.0	228.0	300.0	310.0	208.0	295.0
	315.0	184.0	390.0	310.0	141.0	355.0
1/76	208.0	106.0	185.0	223.0	105.0	200.0
	195.0	115.0	212.0	209.0	95.0	222.0
2/76	256.0	74.0	142.0	231.0	85.0	184.0
	248.0	78.0	166.0	216.0	92.0	123.0
3/76	380.0	193.0	385.0	310.0	174.0	380.0
	375.0	195.0	440.0	300.0	157.0	380.0
4/76	325.0	206.0	216.0	365.0	185.0	265.0
	390.0	256.0	208.0	425.0	252.0	197.0

from 195 in January to 415 in September, with the overall mean value being 319.4. The rapid method likewise gave very similar results with the values ranging from 209 in January to 425 in September, with the overall mean being 317.4.

Washed Oysters Stored at 0°C

The APC values, estimated by the official method, of washed oysters that were stored at 0°C for 7 days (column 2, Table 1) ranged from 76 in October to 256 in November, with the overall mean value being 154.8. The corresponding values for the rapid method (column 5, Table I) ranged from 61 in April to 235 in February, with the overall mean value being 142.2.

Based on the overall mean values, the results of the official method indicated that the APC organisms in washed oysters stored for 7 days at 0°C increased from 87.6 to 154.8, which corresponded to an increase of 77%. Similarly, the results associated with the rapid method indicated an increase in overall mean values from 78.1 to 142.2, which corresponded to an increase of 82$. Increases in the bacterial numbers in oysters during iced storage for a period of 1 week have been reported by Alford et al. (1941), Leibman (1957); Kelly et al. (1961), and Hoff et al. (1967).

After the washed oysters had been stored at 0°C for 14 days, the APC values, whether estimated by the official or rapid method, ranged from approximately 5,000 for oysters harvested in

January to 10,000 for oysters harvested in September. The overall mean value was 8,094 according to the official method and 8,043 according to the rapid method. Both of the microbiological laboratory methods were in close agreement that the overall mean APC values of washed oysters stored at 0°C increased about 80% during the first week of storage, whereas a 50-fold increase occured during the second week of storage. Using prolonged storage times, Alford et al. (1941), Kelly et al. (1961), and Hoff et al. (1967) reported that bacterial numbers in oysters increased greatly after 14 days iced storage.

Washed Oysters Stored at -20°C

The APC values for washed oysters which had been stored at -20°C for 10 days (columns 2 and 5, Table II) were substantially less than the corresponding initial values (column 1, Table II). For the frozen oysters, the official method gave values ranging from 22 January to 56 in September the rapid method gave an almost identical range of values, 21 in January and 53 in September. The overall mean values associated with the official and rapid method were 40.2 and 37.6, respectively. Continued storage of the oysters at -20°C for an additional 10 days restored the values (columns 3 and 6, Table II) to approximately their original levels with the overall mean values after a total of 20 days storage being 82.0 (official) and 74.7 (rapid) as compared to the corresponding initial values of 87.6 and 78.1. These values

were not unexpected because of the effect of freezing temperatures. Kelly et al. (1961), reported a decrease in APC values after 7 days storage at -21°C followed by an increase in APC values after an additional 13 days storage at that temperature.

Unwashed Oysters Stored at 0°C

The APC values of unwashed oysters that were stored at 0°C for 7 days (column 2, Table III), as estimated by the official method, ranged from 315 in October to 2,250 in March, with the overall mean value being 830.0. The range of values associated with the rapid method (column 5, Table III) was from 345 in October to 1,585 in March, with the overall mean value being 808.4. These results show that the overall mean value, associated with the official method using unwashed oysters that were stored 7 days at 0°C, increased from 319.4 to 830.0, which corresponded to an increase of 160%. Similar results were obtained using the rapid method, with the overall mean increasing from 317.4 to 808.4 which corresponded to an increase of 155%.

After the unwashed oysters had been stored 14 days at 0°C, the APC values associated with the official method (column 3, Table III) ranged from 5,400 in February to 45,000 in March, with the overall mean value being 15,941. The corresponding values associated with the rapid method ranged from 7,500 in January to 36,500 in March, with the overall mean value being 16,106. Both of the microbiological

laboratory procedures, as was noted in the experiments using washed oysters, yielded very similar overall mean APC values of unwashed oysters stored at 0°. Both methods indicated an increase of 155 - 160% during the first week of storage, followed during the second week by an extraordinary 20-fold increase.

Unwashed Oysters Stored at -20°C

The APC values of unwashed oysters that had been stored at -20°C for 10 days (columns 2 and 5, Table IV) averaged about 50% less than the corresponding initial values (column 1, Table IV). The values associated with the official method ranged from 74 in February to 256 in April, and those associated with the rapid method ranged from 85 in February to 305 in September. The overall mean values were 170.0 and 171.4 for the official and rapid methods, respectively. After an additional 10-day period of storage of the unwashed oysters at -20°C, the APC values associated with the official method (column 3, Table IV) ranged from 142 in February to 480 in September with the overall mean value being 300.9; the range of values associated with the rapid method (column 6, Table IV) was from 123 in February to 450 in October, with the overall mean being 292.6.

These overall mean APC values for unwashed oysters that were stored at -20°C for 20 days namely, 300.9 (official) and 292.6 (rapid), are slightly less (6-8%) than the corresponding initial values of 319.4 and 317.4.

The ranges and overall means of the foregoing ten pairs of APC values are summarized in Table V.

Effect of Storage Temperature

The storage temperature greatly affected the mean APC values of oysters. An overall mean value of 4,243.5 was associated with oysters stored at 0°C, but that for oysters stored at -20°C was only 164.3 which corresponded to a difference of more than 25-fold. This large difference was expected because of the well-known initially decimating effects of subfreezing temperatures on these organisms.

Effect of Washing Treatment

The washing treatment markedly reduced ($p < 0.01$) the overall mean APC values associated with both iced (0°C) and frozen (-20°C) oysters:

	Storage Temperature	
	0°C	-20°C
Unwashed Oysters:	5,720.4	261.0
Washed Oysters:	2,766.7	66.7

Effect (Reliability) of Laboratory Methodology

Both laboratory methodologies used, rapid and official, demonstrated close agreement on the overall numerical mean APC values associated with iced and frozen oysters. For iced oysters the mean values were 4,237.8 and 4,249.3 for the official and rapid techniques, respectively. The difference, 11.5, corresponded to 0.27% which was not significant. For frozen oysters, the official and rapid techniques gave overall mean APC values of 166.7 and 162.0, respectively.

Table V Summary of Ranges and Overall Means of Aerobic Plate Count Values (x 100) of Oysters Subjected to Various Treatments

(Legend: A = washed oysters; B = unwashed oysters)

	Storage °C	Days	Lab Method	Range (MPN/g) Minimum	Maximum	Overall Mean
A.						
	Initial	0	Official	55.0 Jan	116.0 Apr	87.6
			Rapid	56.0 Jan	114.0 Sep	78.1
	0°C	7	Official	76.0 Oct	256.0 Nov	154.8
			Rapid	61.0 Apr	235.0 Feb	142.2
		14	Official	5100.0 Jan	9900.0 Sep	8094.4
			Rapid	4950.0 Jan	10500.0 Sep	8043.1
	-20°C	10	Official	22.0 Jan	56.0 Sep	40.2
			Rapid	21.0 Jan	53.0 Sep	37.6
		20	Official	57.0 Jan	110.0 Sep	82.0
			Rapid	49.0 Jan	100.0 Sep	74.7
B.						
	Initial	0	Official	195.0 Jan	415.0 Sep	319.4
			Rapid	209.0 Jan	425.0 Sep	317.4
	0°C	7	Official	315.0 Oct	2250.0 Mar	830.0
			Rapid	345.0 Oct	1585.0 Mar	808.4
		14	Official	5400.0 Feb	45000.0 Mar	15940.6
			Rapid	7500.0 Jan	36500.0 Mar	16106.2
	-20°C	10	Official	74.0 Feb	256.0 Apr	170.0
			Rapid	85.0 Feb	305.0 Sep	171.4
		20	Official	142.0 Feb	480.0 Sep	300.9
			Rapid	123.0 Feb	450.0 Oct	292.6

Although the numerical difference between these means was only 4.7, it corresponded to 2.9% which was statistically significant ($p < 0.05$).

Effect of Elapsed Storage Time

Increasing the length of time the oysters were held in cold storage greatly affected ($p < 0.01$) the mean numbers of APC organisms in iced and frozen oysters, whether washed or unwashed. The mean values for iced oysters steadily increased throughout the 14-day storage period, whereas for frozen oysters stored at -20°C, the mean APC values after 10 days of storage had decreased to about half their initial values, but after storage for 20 days at -20°C had returned to about 95% of their initial values. The mean numerical APC values were:

Iced Oysters (0°C) Storage Period (Days)	Treatment	
	Washed	Unwashed
0 (Initial)	82.9	318.4
7	148.5	819.2
14	8,068.8	16,023.4
Frozen Oysters (-20°) Storage Period (Days)		
0 (Initial)	82.9	318.4
10	38.9	170.7
20	78.3	296.8

Effect of Harvest Month

The month in which the oysters were harvested had a very significant effect ($p < 0.01$) on the numbers of APC organisms present in both iced and frozen oysters. The smallest mean monthly values were associated with oysters harvested in January, regardless of the temperature at which they were

stored. The largest mean monthly value for iced oysters, 7,410.2, was associated with samples collected in March, whereas for frozen oysters, the largest mean monthly value, 220.2, was associated with specimens harvested in September. The mean monthly APC values were:

Month	Iced Oysters (0°C)	Frozen Oysters (-20°C)
September	3,622.1	220.2
October	3,114.7	177.7
November	4,975.1	164.2
December	5,288.9	170.7
January	2,436.7	108.9
February	3,489.3	110.7
March	7,410.2	188.9
April	3,611.2	173.4

Effect of the Interaction, Washing Treatment x Laboratory Methodology

For iced oysters stored at 0°C and for frozen oysters stored at -20°C, the bivariate interaction, washing treatment x laboratory methodology, was not significant. The mean APC values were:

Iced Oysters (0°C)	Laboratory Methodology	
	Official	Rapid
Washed	2,778.9	2,754.5
Unwashed	5,696.7	5,744.0
Frozen Oysters (-20°C)		
Washed	69.9	63.5
Unwashed	263.4	260.5

The results of the two laboratory methods were in close

agreement. Washed oysters that had been stored at -20°C were associated with the smallest values; next were the values for unwashed oysters that had been stored at -20°C; then occurred a 10-fold increase in APC values which was associated with washed oysters that had been stored at 0°C; and the largest values were associated with unwashed oysters that had been stored at 0°C. Thus, for both iced and frozen oysters, with respect to their mean APC values, the relationship between washing treatment x laboratory methodology was consistent for all groups of oysters.

In Tables 1 through 10 (Appendix) are listed the detailed results of the analyses of variance of APC values of iced and frozen oysters, together with means for each of the four variables and all their interactions.

Practical Application of the Rapid APC Technique

The obvious advantage of the rapid APC technique is that of time. Because the rapid procedure requires half the time, it would serve as a check on oysters before leaving the plant. It should be noted, however, that though the rapid procedure is statistically comparable to the official technique, it generally produced data indicating a lesser degree of colony production. A detailed comparison of the two techniques showed greater colony production with the official technique in 61 out of 96 cases. The Eugon agar used in the rapid technique supplies the bacteria with a greater concentration of glucose, thus increasing the metabolic rate and

prolonging rapid colony production. However, the nutritionally adequate Eugon agar did not promote as much growth in 24 hours as did the 48-hour official technique using PCA.

The 24-hour APC procedure is not to be recommended as a replacement for the official technique. However, the rapid procedure would be more advantageous in the food industry for routine quality control and process monitoring situations. In addition, the rapid procedure would be a valuable asset in the bacteriological examination of oysters, both in harvesting areas and in processing plants.

II. Coliform Values of Oysters

In Tables VI - IX are presented in chronological sequence for the eight-months period, September, 1975 through April, 1976, the numbers of coliforms found in oysters in duplicate determinations as estimated by each of the two microbiological procedures, rapid and official, which are being compared in these experiments.

In data columns 1 - 3 of these tables are given the values associated with the official method and in columns 4 - 6 are the corresponding values associated with the rapid method. The data obtained for washed oysters are in Tables VI and VII, and those for unwashed oysters are in Tables VIII and IX.

The individual values associated with the official method (expressed as MPN/g) that were obtained for the eight duplicate monthly groups of washed freshly harvested oysters (column 1) during the period, September, 1975 through April, 1976, ranged from 36.0 in January to 95.0 in September, with the overall mean for the sixteen individual values being 60.3. The corresponding values associated with the rapid method (column 4) ranged from 39.0 in January to 93.0 in September, with the overall mean value being 57.7.

For the corresponding unwashed groups of oysters (Tables VIII and IX), the official method gave values ranging from 42.0 in January to 120.0 in September, with the overall mean value being 71.1. The rapid method gave similar results

Table VI Coliform MPN Values of Washed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	95.0	75.0	240.0	93.0	75.0	210.0
	93.0	75.0	290.0	75.0	64.0	160.0
10/75	75.0	75.0	240.0	64.0	53.0	160.0
	53.0	44.0	160.0	75.0	64.0	210.0
11/75	42.0	75.0	95.0	53.0	93.0	120.0
	42.0	53.0	75.0	44.0	64.0	93.0
12/75	44.0	64.0	95.0	42.0	53.0	93.0
	43.0	64.0	64.0	42.0	44.0	75.0
1/76	36.0	75.0	93.0	39.0	64.0	75.0
	42.0	93.0	75.0	43.0	75.0	75.0
2/76	39.0	53.0	64.0	42.0	53.0	64.0
	53.0	64.0	75.0	44.0	53.0	75.0
3/76	64.0	64.0	160.0	53.0	64.0	150.0
	75.0	93.0	160.0	64.0	64.0	120.0
4/76	93.0	75.0	140.0	75.0	64.0	210.0
	75.0	53.0	160.0	75.0	75.0	290.0

Table VII Coliform MPN Values of Washed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	95.0	6.2	3.6	93.0	6.0	3.6
	93.0	6.0	6.0	75.0	3.6	3.0
10/75	75.0	0.0	0.0	64.0	3.0	3.0
	53.0	3.0	0.0	75.0	3.6	3.0
11/75	42.0	3.6	3.0	53.0	3.0	0.0
	42.0	3.0	3.0	44.0	0.0	3.0
12/75	44.0	0.0	0.0	42.0	0.0	0.0
	43.0	3.0	0.0	42.0	3.0	0.0
1/76	36.0	0.0	0.0	39.0	0.0	0.0
	42.0	0.0	0.0	43.0	0.0	0.0
2/76	39.0	0.0	0.0	42.0	3.0	0.0
	53.0	0.0	0.0	44.0	3.0	0.0
3/76	64.0	3.0	3.0	53.0	3.6	0.0
	75.0	0.0	0.0	64.0	3.0	3.6
4/76	93.0	0.0	0.0	75.0	3.0	3.0
	75.0	3.0	0.0	75.0	3.6	3.0

Table VIII Coliform MPN Values of Unwashed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	120.0	120.0	1100.0	95.0	95.0	1100.0
	95.0	93.0	1101.0	75.0	75.0	460.0
10/75	93.0	93.0	460.0	75.0	53.0	210.0
	75.0	75.0	210.0	75.0	75.0	210.0
11/75	53.0	93.0	120.0	53.0	95.0	120.0
	44.0	64.0	93.0	53.0	93.0	95.0
12/75	53.0	75.0	120.0	44.0	53.0	95.0
	64.0	64.0	64.0	53.0	53.0	75.0
1/76	42.0	75.0	160.0	39.0	75.0	95.0
	44.0	95.0	160.0	44.0	75.0	95.0
2/76	53.0	75.0	95.0	53.0	64.0	75.0
	64.0	64.0	75.0	53.0	53.0	93.0
3/76	75.0	75.0	240.0	53.0	75.0	160.0
	75.0	95.0	460.0	75.0	93.0	240.0
4/76	95.0	95.0	290.0	75.0	75.0	210.0
	93.0	64.0	290.0	120.0	93.0	290.0

Table IX Coliform MPN Values of Unwashed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	120.0	9.3	3.6	95.0	9.0	6.1
	95.0	6.0	6.0	75.0	3.6	3.6
10/75	93.0	3.0	0.0	75.0	3.6	3.6
	75.0	6.1	0.0	75.0	6.1	3.0
11/75	53.0	6.1	3.0	53.0	3.6	0.0
	44.0	6.2	3.6	53.0	3.0	3.0
12/75	53.0	0.0	0.0	44.0	3.0	3.0
	64.0	3.0	3.0	53.0	3.6	0.0
1/76	42.0	3.0	0.0	39.0	0.0	0.0
	44.0	0.0	0.0	44.0	0.0	0.0
2/76	53.0	3.0	3.0	53.0	3.6	3.0
	64.0	3.0	3.0	53.0	3.0	0.0
3/76	75.0	3.6	3.0	53.0	6.2	3.0
	75.0	3.0	3.0	75.0	3.6	3.6
4/76	95.0	0.0	0.0	75.0	3.6	3.6
	93.0	3.6	0.0	120.0	3.6	3.6

ranging from 39.0 in January to 120.0 in April, with the overall mean being 64.7.

Washed Oysters Stored at 0°C

The coliform values, estimated by the official method, of washed oysters that were stored at 0°C for 7 days (column 2, Table VI) ranged from 44.0 in October to 93.0 in January, with the overall mean value being 68.4. The corresponding values for the rapid method (column 5, Table VI), ranged from 44.0 in December to 75.0 in September, with the overall mean value being 63.9.

Based on the overall mean values, the results of the official method indicated that the coliform organisms in washed oysters stored for 7 days at 0°C increased from 60.3 to 68.4, which corresponded to an increase of 13%. Increases in coliform numbers in oysters during iced storage have been reported by Tonney and White (1926), Wilson and McCleskey (1951), Kelly (1961) Hoff et al. (1967) and Murchelano et al. (1975). Similarly, the results associated with the rapid method indicated an increase in overall mean values from 57.7 to 63.9, which corresponded to an increase of 11%.

After the washed oysters had been stored at 0°C for 14 days, the coliform values, whether estimated by the official or rapid methods, ranged from approximately 60.0 for oysters harvested in December to 300.0 for oysters harvested in September. The overall mean value was 142.9 according to the official method and 136.3 according to the rapid method. Both of the microbiological laboratory methods employed

were in close agreement that the overall mean coliform values of washed oysters stored at 0°C increased about 12% during the first week of storage whereas a 2-fold increase occurred during the second week of storage. Kelly (1961) and Hoff et al. (1967) reported that coliform numbers increased after 14 days storage in ice.

Washed Oysters Stored at -20°C

The coliform values of washed oysters which had been stored at -20°C for 10 days (columns 2 and 5, Table VII) were much less than the corresponding initial values (column 1, Table VII). For the frozen oysters the official method gave values ranging from 0.0 in January to 6.2 in September. The overall mean values associated with the official and rapid methodswere 1.9 and 2.6, respectively. Continued storage of the oysters at -20°C for an additional 10 days decreased the values (columns 3 and 6, Table VII), with the overall mean values after a total of 20 days storage being 1.2 (official) and 1.6 (rapid). Similar results were reported by Kelly et al. (1961). Reports of decreases in bacterial numbers at low holding temperatures were reported by Tressler (1937), Burton (1949), Ingraham (1958), and Tennant (1955).

Unwashed Oysters Stored at 0°C

The coliform values of unwashed oysters that were stored at 0°C for 7 days (column 2, Table VIII), as estimated by the official method ranged from 64.0 in November to 120.0 in September, with the overall mean value being 82.2. The

range of values associated with the rapid method (column 5, Table VIII) was from 53.0 in October to 95.0 in September, with the overall mean value being 74.7. These results associated with the official method show that the overall mean value associated with unwashed oysters that were stored 7 days at 0°C increased from 71.1 to 82.2 which corresponded to an increase of 16%. Similar results were obtained using the rapid method, with the overall mean increasing from 64.7 to 74.7 which corresponded to an increase of 15%.

After the unwashed oysters had been stored 14 days at 0°C, the coliform values associated with the official method (column 3, Table VIII) ranged from 64.0 in December to 1101.0 in September, with the overall mean value being 314.9. The corresponding values associated with the rapid method ranged from 75.0 in February to 1100.0 in September, with the overall mean value being 226.4. Both of the microbiological laboratory procedures, as was noted in the experiments using washed oysters, yielded very similar overall mean coliform values of unwashed oysters stored at 0°C. Both methods indicated an increase of 15-16% during the first week of storage, followed during the second week by an average of 18-fold increase.

Unwashed Oysters Stored at -20°C

The coliform values of unwashed oysters that had been stored at -20°C for 10 days (columns 2 and 5, Table IX) were less than the corresponding initial values (column 1, Table IX). The values associated with the official method ranged

from 0.0 in December to 9.3 in September, and those associated with the rapid method ranged from 0.0 in January to 9.0 in September. The overall mean values were 3.7 and 3.8 for the official and rapid methods, respectively. After an additional 10-day period of storage of the unwashed oysters at -20°C, the coliform values associated with the official method (column 3, Table IX) ranged from 0.0 in October to 6.0 in September, with the overall mean value being 2.0; the range of values associated with the rapid method (column 6, Table IX) was from 0.0 in November to 6.1 in September, with the overall mean being 2.4.

These overall mean coliform values for unwashed oysters that were stored 20 days at -20°C, namely 2.0 (official) and 2.4 (rapid), are much less (96-97%) than the corresponding initial values of 71.1 and 64.7.

The ranges and overall means of the foregoing ten pairs of coliform values are summarized in Table X below.

Effect of Storage Temperature

The storage temperature greatly affected the total coliform values of the oysters. An overall mean value of 113.6 was associated with oysters stored at 0°C, but that for oysters stored at -20°C was only 22.7, which corresponded to a difference of more than 5-fold. This large variation in coliform values were expected due to the difference in storage temperatures.

Table X Summary of Ranges and Overall Means of Coliform MPN Values of Oysters Subjected to Various Treatments

(Legend: A = washed oysters; B = unwashed oysters)

	Storage °C	Days	Lab Method	Range (MPN/g) Minimum	Range (MPN/g) Maximum	Overall Mean
A.						
	Initial	0	Official	36.0 Jan	95.0 Sep	60.3
			Rapid	39.0 Jan	93.0 Sep	57.7
	0°C	7	Official	44.0 Oct	93.0 Jan	68.4
			Rapid	44.0 Dec	75.0 Sep	63.9
		14	Official	64.0 Feb	290.0 Sep	136.6
			Rapid	64.0 Feb	290.0 Apr	136.3
	-20°C	10	Official	0.0 Jan Feb	6.2 Sep	1.9
			Rapid	0.0 Jan	6.0 Sep	2.6
		20	Official	0.0 Oct Dec Jan Feb Apr	6.0 Sep	1.2
			Rapid	0.0 Dec Jan Feb	3.6 Sep	1.6
B.						
	Initial	0	Official	42.0 Jan	120.0 Sep	71.1
			Rapid	39.0 Jan	120.0 Apr	64.7
	0°C	7	Official	64.0 Feb	120.0 Sep	82.2
			Rapid	53.0 Dec	95.0 Nov	74.7
		14	Official	64.0 Dec	1101.0 Sep	314.9
			Rapid	75.0 Feb	1100.0 Sep	226.4
	-20°C	10	Official	0.0 Dec Jan	9.3 Sep	3.7
			Rapid	0.0 Jan	9.0 Sep	3.8
		20	Official	0.0 Oct Jan Apr	6.0 Sep	2.0
			Rapid	0.0 Jan	6.1 Sep	2.4

Effect of Washing Treatment.

The washing treatment reduced ($p < 0.05$) the overall mean total coliform values associated with both iced (0°C) and frozen (-20°C) oysters:

	Storage Temperature	
	0°C	-20°C
Unwashed Oysters	139.0	24.6
Washed Oysters	88.2	20.9

Effect (Reliability) of Laboratory Methodology

Both laboratory methodologies used, rapid and official, demonstrated close agreement on the overall numerical mean total coliform values associated with iced and frozen oysters. For iced oysters the mean values were 123.3 and 103.9 for the official and rapid techniques, respectively. The difference, 19.4 corresponded to 1.6% which was statistically significant ($p < 0.05$). For frozen oysters, the official and rapid techniques gave overall mean total coliform values of 23.3 and 22.1, respectively. The numerical difference between these means, 1.2, corresponded to 0.05% which was not significantly different.

Effect of Elapsed Storage Time

Increasing the length of time the oysters were held in cold storage greatly affected ($p < 0.01$) the mean numbers of total coliform organisms in iced and frozen oysters, whether washed or unwashed. The mean values for iced oysters throughout the 14-day storage period, whereas for frozen

oysters stored at -20°C, the mean total coliform values after 10 days of storage had decreased about 25-fold, and after storage for 20 days at -20°C had further decreased. The mean numerical total coliform values were:

Iced Oysters (0°C) Storage Period (Days)	Treatment Washed	Unwashed
0 (Initial)	59.0	67.9
7	66.2	78.4
14	139.6	270.7
Frozen Oysters (-20°C) Storage Period (Days)		
0 (Initial)	59.0	67.9
10	2.3	3.7
20	1.4	2.2

Effect of Harvest Month

The month in which the oysters were harvested had a very significant effect ($p < 0.01$) on the numbers of total coliform organisms present in both iced and frozen oysters. The smallest mean monthly value for iced oysters, 62.3, was associated with oysters collected in February, whereas for frozen oysters, the smallest mean monthly value 13.8, was associated with oysters harvested in January. The largest mean monthly values were associated with oysters harvested in September, regardless of the temperature at which they were stored. The mean monthly total coliform values were:

Month	Iced Oysters (0°C)	Frozen Oysters (-20°C)
September	253.1	34.4
October	124.0	26.1

November	76.0	18.0
December	64.0	16.9
January	74.3	13.8
February	62.3	17.9
March	118.6	24.1
April	136.4	30.6

Effect of Interaction, Washing Treatment x Laboratory Methodology

For iced oysters stored at 0°C, the bivariate interaction, washing treatment x laboratory methodology, was significant ($p < 0.05$) whereas for frozen oysters, this interaction was not statistically significant. The mean total coliform values were:

Iced Oysters (0°C)	Laboratory Methodology	
	Official	Rapid
Washed	90.5	85.9
Unwashed	156.1	121.9
Frozen Oysters (-20°C)		
Washed	21.1	20.6
Unwashed	25.6	23.6

The results of the two laboratory methods were in close agreement. Washed oysters that had been stored at -20°C were associated with the smallest values; next were the values for unwashed oysters that had been stored at -20°C; then occurred a 3.6-fold increase in total coliform values which was associated with washed oysters at 0°C; and the largest values were associated with unwashed oysters that had been stored at 0°C.

Thus, the relationship betwen washing treatment and laboratory methodology was consistent for groups of frozen oysters only.

In Tables 11 through 20 (Appendix) are listed the detailed results of the analysis of variance of total coliform values of iced and frozen oysters, together with means for each of the four variables and their interactions.

Practical Application of the Rapid Total Coliform Technique.

The advantage of the rapid total coliform procedures is the requirement of only 24 hours. The rapid technique would prove to be a valuable asset in the bacteriological examination of oysters both in harvesting areas and processing plants. The rapid procedure would also be advantageous in the application of quality control and process monitoring.

The rapid coliform procedure is not intended to substitute for the official procedure. The additional steps of the official technique eliminate inherent errors in the MPN procedure.

III. Escherichia coli Values of Oysters

In Tables XI - XIV are presented in chronological sequence for the eight months period, September, 1975 through April, 1976, the numbers of E. coli organisms found in oysters in duplicate determinations as estimated by each of the two microbiological procedures, rapid and official standard, which are being compared in these experiments.

Table XI *Escherichia coli* MPN Values of Washed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	13.0	9.0	9.0	11.0	7.2	9.0
	11.0	9.1	9.1	9.4	7.2	9.2
10/75	11.0	9.3	9.3	9.4	9.2	11.0
	7.2	7.2	9.4	9.3	7.2	9.3
11/75	7.2	9.0	6.1	6.2	7.2	3.0
	6.1	7.2	3.0	7.2	9.0	3.0
12/75	3.0	3.0	0.0	3.6	3.6	0.0
	3.0	3.0	0.0	3.0	0.0	0.0
1/76	3.0	3.0	3.0	3.6	3.6	3.0
	3.0	0.0	0.0	3.6	3.6	3.0
2/76	3.0	0.0	3.0	3.0	3.0	0.0
	6.0	3.0	3.0	3.0	0.0	0.0
3/76	9.0	9.0	9.3	7.2	9.0	11.0
	9.4	11.0	11.0	9.2	9.4	11.0
4/76	9.0	3.6	6.0	9.0	6.0	6.1
	9.2	3.0	3.6	9.1	6.0	6.0

Table XII *Escherichia coli* MPN Values of Washed Oysters Stored at -20°C using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	13.0	3.6	0.0	11.0	3.6	3.0
	11.0	3.0	0.0	9.4	3.6	0.0
10/75	11.0	0.0	0.0	9.4	0.0	0.0
	7.2	3.0	3.0	9.3	0.0	0.0
11/75	7.2	3.0	0.0	6.2	3.0	3.0
	6.1	3.0	0.0	7.2	0.0	0.0
12/75	3.0	0.0	0.0	3.6	0.0	0.0
	3.0	0.0	0.0	3.0	0.0	0.0
1/76	3.0	0.0	0.0	3.6	0.0	0.0
	3.0	0.0	0.0	3.6	0.0	0.0
2/76	3.0	0.0	0.0	3.0	0.0	0.0
	6.0	0.0	0.0	3.0	0.0	0.0
3/76	9.0	3.0	0.0	7.2	3.6	0.0
	9.4	0.0	0.0	9.2	3.0	3.0
4/76	9.0	0.0	0.0	9.0	3.0	0.0
	9.2	0.0	0.0	9.1	0.0	0.0

Table XIII *Escherichia coli* MPN Values of Unwashed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	13.0	9.4	9.3	13.0	12.0	9.2
	11.0	9.1	9.2	13.0	12.0	9.4
10/75	11.0	9.3	9.4	9.4	9.3	12.0
	9.0	9.0	9.4	9.4	9.1	9.3
11/75	7.2	9.1	6.2	7.2	7.2	3.6
	7.2	9.0	9.1	9.0	9.0	7.2
12/75	3.6	6.1	0.0	3.6	3.6	3.0
	7.2	7.2	6.1	3.6	3.0	3.0
1/76	3.6	3.6	3.0	6.1	3.6	3.6
	3.0	3.0	3.0	3.6	3.6	3.6
2/76	6.1	3.0	3.0	3.6	3.6	3.6
	7.2	6.0	3.0	6.2	3.0	6.0
3/76	11.0	9.0	12.0	7.2	9.1	11.0
	13.0	11.0	11.0	12.0	12.0	11.0
4/76	9.2	9.4	9.0	9.0	9.0	7.2
	9.2	9.2	7.2	9.2	11.0	9.3

Table XIV *Escherichia coli* MPN Values of Unwashed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	13.0	6.1	3.0	13.0	3.6	3.6
	11.0	3.0	0.0	13.0	7.2	3.0
10/75	11.0	3.6	3.0	9.4	3.6	3.6
	9.0	3.6	0.0	9.4	3.6	3.6
11/75	7.2	3.0	0.0	7.2	3.6	3.0
	7.2	6.1	3.0	9.0	3.0	3.0
12/75	3.6	0.0	0.0	3.6	3.0	3.0
	7.2	0.0	0.0	3.6	3.0	0.0
1/76	3.6	3.0	3.0	6.1	0.0	0.0
	3.0	3.0	0.0	3.6	0.0	0.0
2/76	6.1	0.0	0.0	3.6	3.0	0.0
	7.2	3.0	3.0	6.2	3.0	0.0
3/76	11.0	6.1	6.0	7.2	3.6	3.6
	13.0	3.0	3.0	12.0	6.1	3.6
4/76	9.2	3.0	0.0	9.0	3.0	3.0
	9.2	0.0	0.0	9.2	3.6	0.0

In data columns 1 - 3 of the tables are given the values associated with the official method and in columns 4 - 6 are the corresponding values associated with the rapid method. The data obtained for washed oysters are in Tables XI and XII, and those for unwashed oysters are in Tables XIII and XIV.

The individual values associated with the official method (expressed as MPN/g) that were obtained for the eight duplicate monthly groups of washed freshly harvested oysters (column 1) during the period, September, 1975 through April, 1976, ranged from 3.0 in December to 13.0 in September, with the overall mean for the 16 individual values being 7.1. The corresponding values associated with the rapid method (column 4) ranged from 3.0 in December to 11.0 in September, with the overall mean value being 6.7.

For the corresponding unwashed group of oysters (Tables XIII and XIV), the official method gave values ranging from 3.0 in January to 13.0 in September with the overall mean value being 8.2. The rapid method gave similar results with the values ranging from 3.6 in December to 13.0 in September, with the overall mean being 7.8.

Washed Oysters Stored 0°C.

The E. coli values, estimated by the official method, of washed oysters that were stored at 0°C for 7 days (column 2, Table XI) ranged from 0.0 in January to 11.0 in March, with the overall mean value being 5.6. The corresponding

values for the rapid method (column 5, Table XI), ranged from 0.0 in February to 9.4 in March, with the overall mean value being 5.7.

Based on the overall mean values, the results of the official method indicated that the E. coli organisms in washed oysters stored for 7 days at 0°C decreased from 7.1 to 5.6, which corresponded to a decrease of 21%. Decreases in E. coli numbers in oysters during iced storage have been reported by Hunter and Harrison (1928), Wilson and McCleskey (1951), Kelly (1961), and Hoff et al. (1967). Similarly, the results associated with the rapid method indicated a decrease in overall mean values from 6.7 to 5.7, which corresponded to a decrease of 15%.

After the washed oysters had been stored at 0°C for 14 days, the E. coli values, whether estimated by the official or rapid methods, ranged from 0.0 for oysters harvested in December to 11.0 for oysters harvested in March. The overall mean value was 5.3 according to the official and rapid methods. Both of the microbiological laboratory methods were in close agreement that the overall mean E. coli values of washed oysters stored at 0°C decreased about 20% during the first week of storage whereas a 1.3-fold decrease occurred during the second week of storage. Using prolonged storage times, Wilson and McCleskey (1951), Kelly (1961) and Hoff et al. (1967) reported that numbers of E. coli decreased after 14 days storage in ice.

Washed Oysters Stored at -20°C

The E. coli values of washed oysters which had been stored at -20°C for 10 days (columns 2 and 5, Table XII) were much less than the corresponding initial values (column 1, Table XII). For the frozen oysters, the official method gave values ranging from 0.0 in December to 3.6 in September; the rapid method gave an almost identical range of values, 0.0 in December to 3.6 in September. The overall mean values associated with the official and rapid methods were both 1.2. Continued storage of the oysters at -20°C for an additional 10 days decreased the value (columns 3 and 6, Table XII) with the overall mean values after a total of 20 days storage being 0.2 (official) and 0.6 (rapid). Decreases in E. coli values after several days storage at freezing temperature were also reported by Burton (1949), Ingraham (1958) and Kelly et al. (1961).

Unwashed Oysters Stored at 0°C

The E. coli values of unwashed oysters that were stored at 0°C for 7 days (column 2, Table XIII), as estimated by the official method, ranged from 3.0 in January to 11.0 in March, with the overall mean value being 7.7 The range of values associated with the rapid method (column 5, Table XIII) was from 3.0 in December to 12.0 in September, with the overall mean value being 7.5. These results associated with the official method show that the overall mean value associated with unwashed oysters that were stored 7 days at 0°C decreased from 8.2 to 7.7 which corresponded to a decrease of 6%.

Similar results were obtained using the rapid method, with the overall mean decreased from 7.8 to 7.5 which corresponded to a decrease of 4%.

After the unwashed oysters had been stored 14 days at 0°C, the E. coli values associated with the official method (column 3, Table XIII) ranged from 0.0 in December to 12.0 in March, with the overall mean value being 6.9. The corresponding values associated with the rapid method ranged from 3.0 in December to 12.0 in October, with the overall mean value being 7.0. Both of the microbiological laboratory procedures, as was noted in the experiments using washed oysters, yielded very similar overall mean E. coli values of unwashed oysters stored at 0°C. Both methods indicated a decrease of 4 - 6% during the first week of storage, followed during the second week by a 2.5-fold decrease.

Unwashed Oysters Stored at -20°C

The E. coli values of unwashed oysters that had been stored at -20°C for 10 days (columns 2 and 5, Table XIV) were less than the corresponding initial values (column 1, Table XIV). The values associated with the official method ranged from 0.0 in December to 6.1 in September; and those associated with the rapid method ranged from 0.0 in January to 7.2 in September. The overall mean values were 2.9 and 3.3 for the official and rapid methods, respectively. After an additional 10 day period of storage of the unwashed oysters at -20°C, the values associated with the official method (column 3, Table XIV) ranged from 0.0 in December to 6.0 in March, with

the overall mean value being 1.5; the range of values associated with the rapid method (column 6, Table XIV) was from 0.0 in February to 3.6 in September, with the overall mean being 2.0.

The overall mean E. coli values for unwashed oysters that were stored 20 days at -20°C, namely 1.5 (official) and 2.0 (rapid), are much less (74 - 82%) than the corresponding initial values of 8.2 and 7.8.

The ranges and overall means of the foregoing ten pairs of E. coli values are summarized in Table XV.

Effect of Storage Temperature

The storage temperature greatly affected the E. coli values of the oysters. The mean value of 6.7 was associated with oysters stored at 0°C, but that for oysters stored at -20° was 3.6, which corresponded to a difference of more than 2-fold. This difference in E. coli values was expected due to the differing storage temperatures.

Effect of Washing Treatment

The washing treatment greatly reduced ($p < 0.01$) the overall mean E. coli values associated with both iced (0°C) and frozen (-20°C) oysters:

	Storage Temperature	
	0°C	-20°C
Unwashed Oysters	7.4	4.3
Washed Oysters	5.9	2.8

Table XV Summary and Ranges and Overall Means of *Escherichia coli* MPN Values of Oysters Subjected to Various Treatments

(Legend: A = washed oysters; B = unwashed oysters)

	Storage °C	Days	Lab Method	Range (MPN/g) Minimum	Range (MPN/g) Maximum	Overall Mean
A.						
	Initial	0	Official	3.0 Dec Jan	13.0 Sep	7.1
			Rapid	3.0 Feb	11.0 Sep	6.7
	0°C	7	Official	0.0 Jan Feb	11.0 Mar	5.6
			Rapid	0.0 Feb	9.4 Mar	5.7
		14	Official	0.0 Dec	11.0 Mar	5.3
			Rapid	0.0 Dec Feb	11.0 Mar	5.3
	-20°C	10	Official	0.0 Dec Jan Feb Apr	3.6 Sep	1.2
			Rapid	0.0 Oct Dec Jan Feb	3.6 Sep	1.2
		20	Official	0.0 Sep Nov Dec Jan Feb Mar	3.0 Oct	0.2
			Rapid	0.0 Oct Dec Jan Feb Apr	3.0 Sep Mar	0.6
B.						
	Initial	0	Official	3.0 Jan	13.0 Sep Mar	8.2
			Rapid	3.6 Dec	13.0 Sep	7.8
	0°C	7	Official	3.0 Jan	11.0 Mar	7.7
			Rapid	3.0 Dec Feb	12.0 Sep	7.5
		14	Official	0.0 Dec	12.0 Mar	6.9
			Rapid	3.0 Dec	12.0 Oct	7.0
	-20°C	10	Official	0.0 Dec	6.1 Sep Mar	2.9
			Rapid	0.0 Jan	7.2 Sep	3.3
		20	Official	0.0 Dec Apr	6.0 Mar	1.5
			Rapid	0.0 Jan Feb	3.6 Sep Mar	2.0

Effect (Reliability) of Laboratory Methodology

Both laboratory methodologies used, rapid and official, demonstrated close agreement on the overall numerical mean E. coli values associated with iced and frozen oysters. For iced oysters, the mean values were 6.8 and 6.7 for official and rapid techniques, respectively. The difference, 0.1, was not statistically significant. For frozen oysters, the official and rapid techniques gave overall mean E. coli values of 3.5 and 3.6, respectively. The numerical difference between these means, 0.1, was also not statistically significant.

Effect of Elapsed Storage Time

Increasing the length of time the oysters were held in cold storage greatly affected ($p < 0.01$) the mean numbers of E. coli organisms in iced and frozen oysters, whether washed or unwashed. The mean values for iced and frozen oysters decreased throughout the storage periods. The mean numerical E. coli values were:

Iced Oysters (0°C) Storage Period (Days)	Treatment	
	Washed	Unwashed
0 (Initial)	6.9	8.0
7	5.6	7.6
14	5.3	6.9
Frozen Oysters (-20°C) Storage Period (Days)		
0 (Initial)	6.9	8.0
10	1.2	3.1
20	0.4	1.7

Effect of Harvest Month

The month in which the oysters were harvested had a very significant effect ($p < 0.01$) on the numbers of E. coli organisms present in both iced and frozen oysters. The smallest mean monthly values for iced oysters, 3.0, was associated with oysters collected in December, whereas for frozen oysters, the smallest mean monthly value, 1.6, was associated with oysters harvested in January. The largest mean monthly values for iced oysters, 10.2, was associated with oysters collected in March, whereas for frozen oysters, the largest mean monthly value, 5.9, was associated with oysters harvested in September. The mean monthly E. coli values were:

Month	Iced Oysters (0°C)	Frozen Oysters (-20°C)
September	10.1	5.9
October	9.4	4.4
November	6.9	3.9
December	3.0	1.7
January	3.2	1.6
February	3.9	2.1
March	10.2	5.2
April	7.7	3.7

Effect of Interaction, Washing Treatment x Laboratory Methodology

For iced oysters stored at 0°C and frozen oysters stored at -20°C, the bivariate interaction, washing treatment x laboratory methodology was not significant. The mean

E. coli values were:

Iced Oysters (0°C)	Laboratory Methodology	
	Official	Rapid
Washed	6.0	5.9
Unwashed	7.6	7.4
Frozen Oysters (-20°C)		
Washed	2.8	2.8
Unwashed	4.2	4.4

The results of the two laboratory methods were in close agreement. Washed oysters that had been stored at -20°C were associated with the smallest values; next were the values for unwashed oysters that had been stored at -20°C; then a 1.5 fold increase in E. coli values which was associated with washed oysters stored at 0°C; and the largest values were associated with unwashed oysters that had been stored at 0°C. Thus, the relationship between washing treatment and laboratory methodology was consistent for all groups of oysters.

In Tables 21 - 30 (Appendix) are listed the detailed results of the analysis of variance of E. coli values of iced and frozen oysters, together with means for each of the four variables and their interactions.

Practical Application of the Rapid Escherichia coli Technique

The rapid technique investigated for the enumeration of E. coli was identical to that suggested by Fisbein et al. (1967) and adopted by official agencies (U. S. Dept. of Health, Education and Welfare, 1969) as a rapid but not standard

procedure. The technique requires only 24 hours but is not as exacting as recommended procedures which require primary and secondary MPN procedures as well as final confirmation on a differential medium.

IV. Coagulase-Positive Staphylococci Values of Oysters

In Tables XVI - XIX are presented in chronological sequence for the eight months period, September, 1975 through April, 1976, the numbers of coagulase-positive staphylococci organisms found in oysters in duplicate determinations as estimated by each of the two microbiological procedures, rapid and official standard, which are being compared in these experiments.

In data columns 1 - 3 of these tables are given the values associated with the official method and in columns 4 - 6 are the corresponding values associated with the rapid method. The data obtained for washed oysters are in Tables XVI and XVII, and those for unwashed oysters are in Tables XVIII and XIX.

The individual values associated with the official method (expressed as MPN/g) that were obtained for the eight duplicate monthly groups of washed freshly harvested oysters (column 1) during the period, September, 1975 through April, 1976, ranged from 0.0 in November to 3.6 in February, with the overall mean for the 16 individual values being 0.8. The corresponding values associated with the rapid method (column 4) ranged from 0.0 in November to 3.6 in September, with the

Table XVI Coagulase-Positive Staphylococci MPN Values of Washed Oysters stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	0.0	0.0	0.0	3.0	3.0	3.0
	0.0	0.0	0.0	3.6	0.0	0.0
10/75	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
11/75	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
12/75	0.0	0.0	0.0	3.6	3.0	3.0
	3.0	0.0	0.0	3.0	3.0	0.0
1/76	0.0	0.0	0.0	3.0	3.0	0.0
	3.0	0.0	0.0	0.0	0.0	0.0
2/76	3.6	3.0	3.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
3/76	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
4/76	3.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0

Table XVII Coagulase-Positive Staphylococci MPN Values of Washed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	0.0	0.0	0.0	3.0	0.0	0.0
	0.0	0.0	0.0	3.6	0.0	0.0
10/75	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	3.0	0.0
11/75	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
12/75	0.0	0.0	0.0	3.6	3.0	0.0
	3.0	0.0	0.0	3.0	0.0	0.0
1/76	0.0	0.0	0.0	3.0	0.0	0.0
	3.0	0.0	0.0	0.0	0.0	0.0
2/76	3.6	3.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
3/76	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
4/76	3.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0

Table XVIII Coagulase-Positive Staphylococci MPN Values of Unwashed Oysters Stored at 0°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	7 Days	14 Days	Rapid Method (MPN/g) Upon Receipt	7 Days	14 Days
9/75	6.1	3.6	3.0	3.0	3.0	3.0
	6.0	3.0	0.0	6.0	3.0	3.0
10/75	0.0	0.0	3.0	0.0	0.0	0.0
	6.1	6.1	3.0	3.0	0.0	0.0
11/75	0.0	0.0	0.0	3.0	3.0	3.0
	0.0	0.0	0.0	0.0	0.0	0.0
12/75	6.1	6.0	3.6	6.1	6.1	3.0
	6.1	3.0	3.0	3.6	3.6	3.0
1/76	3.0	3.0	0.0	3.0	3.6	3.0
	3.0	0.0	0.0	3.0	3.0	0.0
2/76	3.6	3.0	3.0	3.0	3.0	0.0
	3.0	0.0	0.0	6.1	3.0	3.0
3/76	3.6	3.0	3.0	3.0	3.0	3.0
	6.1	3.6	0.0	0.0	0.0	0.0
4/76	6.1	3.0	0.0	3.6	3.0	3.0
	3.0	0.0	0.0	0.0	0.0	0.0

Table XIX Coagulase-Positive Staphylococci MPN Values of Unwashed Oysters Stored at -20°C Using Official and Rapid Methods

Month/ Year	Official Method (MPN/g) Upon Receipt	10 Days	20 Days	Rapid Method (MPN/g) Upon Receipt	10 Days	20 Days
9/75	6.1	3.0	0.0	3.0	3.0	0.0
	6.0	0.0	0.0	6.0	0.0	0.0
10/75	0.0	0.0	0.0	0.0	3.0	0.0
	6.1	3.0	0.0	3.0	0.0	0.0
11/75	0.0	0.0	0.0	3.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
12/75	6.1	0.0	0.0	6.1	0.0	0.0
	6.1	0.0	0.0	3.6	3.0	0.0
1/76	3.0	0.0	0.0	3.0	3.0	0.0
	3.0	0.0	0.0	3.0	0.0	0.0
2/76	3.6	3.0	0.0	3.0	3.6	0.0
	3.0	0.0	0.0	6.1	3.0	0.0
3/76	3.6	0.0	0.0	3.0	0.0	0.0
	6.1	0.0	0.0	0.0	0.0	0.0
4/76	6.1	0.0	0.0	3.6	3.0	0.0
	3.0	0.0	0.0	0.0	0.0	0.0

overall mean value being 1.0.

For the corresponding unwashed groups of oysters (Tables XVIII and XIX), the official method gave values ranging from 0.0 in October to 6.1 in September with the overall mean value being 3.9. The rapid method gave similar results with the values ranging from 0.0 in October to 6.1 in December, with the overall mean being 2.9.

Washed Oysters Stored at 0°C

The coagulase-positive staphylococci values, estimated by the official method, of washed oysters that were stored at 0°C for 7 days (column 2, Table XVI) ranged from 0.0 in all months to 3.0 in February, with the overall mean value being 0.2. The corresponding values for the rapid method (column 5, Table XVI) ranged from 0.0 in November to 3.0 in September, with the overall mean value being 0.8.

Based on the overall mean values, the results of the official method indicated that the coagulase-positive staphylococci organisms in washed oysters stored for 7 days at 0°C decreased from 0.8 to 0.2, which corresponded to a decrease of 75%.

The results associated with the rapid method indicated a decrease in overall mean values from 1.0 to 0.8, which correspond to a decrease of 20%.

After the washed oysters had been stored at 0°C for 14 days, the coagulase-positive staphylococci values, whether estimated by the official or rapid methods, ranged from 0.0

for oysters harvested in all months to 3.0 for oysters harvested in February. The overall mean value was 0.2 according to the official method and 0.4 according to the rapid method. Both of the microbiological laboratory methods were in close agreement that the overall mean coagulase-positive staphylococci values of washed oysters stored at 0°C decreased about 50% during the first week of storage whereas a 1-fold decrease occurred during the second week of storage. No reported data has been found in the literature demonstrating the effect of storage temperature and time on the number of coagulase-positive staphylococci.

Washed Oysters Stored at -20°C

The coagulase-positive staphylococci values of washed oysters which had been stored at -20°C for 10 days (columns 2 and 5, Table XVII) were about the same as the initial values (column 1, Table XVII). For the frozen oysters, the official gave values ranging from 0.0 in January to 3.0 in February; the rapid method gave an identical range of values. The overall mean values associated with the official and rapid methods were 0.2 and 0.4, respectively. Continued storage of the oysters at -20°C for an additional 10 days gave the same value, (column 3 and 6, Table XVII), with the overall mean values after a total of 20 days storage being 0.0 (official) and 0.0 (rapid).

Unwashed Oysters Stored at 0°C

The coagulase-positive staphylococci values of unwashed oysters that were stored at 0°C for 7 days (column 2, Table XVIII), as estimated by the official method, ranged from 0.0 in November to 6.1 in October, with the overall mean value being 2.3. The range of values associated with the rapid method (column 5, Table XVIII) was from 0.0 in October to 6.1 in December, with the overall mean value being 2.3. These results associated with the official method show that the overall mean value associated with unwashed oysters that were stored 7 days at 0°C decreased form 3.9 to 2.3 which corresponded to a decrease of 41%. Similar results were obtained using the rapid method, with the overall mean decreased from 2.9 to 2.3 which corresponded to a decrease of 21%.

After the unwashed oysters had been stored 14 days at 0°C the APC values associated with the official method (column 3, Table XVIII) ranged from 0.0 in January to 3.6 in December, with the overall mean values associated with the rapid method ranged from 0.0 in October to 3.0 in September, with the overall mean value being 1.7. Both of the microbiological laboratory procedures, as was noted in the experiments using washed oysters, yielded very similar overall mean APC values of unwashed oysters stored at 0°C. Both methods indicated a decrease of 21 - 41% during the first week of storage, followed during the second week by a 2-fold decrease.

Unwashed Oysters Stored at -20°C

The values of unwashed oysters that had been stored at -20°C for 10 days (columns 2 and 5, Table XIX) were less than the corresponding initial values (column 1, Table XIX). The values associated with the official method ranged from 0.0 in November to 3.0 in September, and those associated with the rapid method ranged from 0.0 in November to 3.6 in February. The overall mean values were 0.6 and 1.4 for the official and rapid methods, respectively. After an additional 10 day period of storage of the unwashed oysters at -20°C, all monthly values associated with the official and rapid methods, (columns 3 and 6, Table XIX), were 0.0, with the overall mean value being 0.0.

These overall mean coagulase-positive staphylococci values for unwashed oysters that were stored 20 days at -20°C, namely 0.0 (official) and 0.0 (rapid), are extremely lower (100%) than the corresponding initial values of 3.9 and 2.9.

The ranges and overall means of the foregoing ten pairs of coagulase-positive staphylococci values are summarized in Table XX.

Effect of Storage Temperature

The storage temperature greatly affected the coagulase-positive staphylococci values of the oysters. A mean value of 1.5 was associated with oysters stored at 0°C, but that for oysters stored at -20°C was 1.0, which corresponded to a difference of 50%.

Table XX Summary of Ranges and Overall Means of Coagulase-Positive Staphylococci MPN Values of Oysters Subjected to Various Treatments

(Legend: A = washed oysters; B = unwashed oysters)

	Storage 0°C	Days	Lab Method	Range (MPN/g) Minimum	Range (MPN/g) Maximum	Overall Mean
A.						
	Initial	0	Official	0.0 Sep Oct Nov Mar	3.6 Feb	0.8
			Rapid	0.0 Oct Nov Feb Mar Apr	3.6 Sep Dec	1.0
	0°C	7	Official	0.0 Sep Oct Nov Dec Jan Mar Apr	3.0 Feb	0.2
			Rapid	0.0 Oct Nov Feb Mar Apr	3.0 Dec	0.8
		14	Official	0.0 Sep Oct Nov Dec Jan Mar Apr	3.0 Feb	0.2
			Rapid	0.0 Oct Nov Jan Feb Mar Apr	3.0 Sep Dec	0.4
	-20°C	10	Official	0.0 Sep Oct Nov Dec Jan Apr Mar	3.0 Feb	0.2
			Rapid	0.0 Sep Nov Jan Feb Mar Apr	3.0 Oct Dec	0.4
		20	Official	0.0 Sep Oct Nov Dec Jan Feb Mar Apr	0.0	0.0
			Rapid	0.0 Sep Oct Nov Dec Jan Feb Mar Apr	0.0	0.0
B.						
	Initial	0	Official	0.0 Nov	6.1 Dec	3.9
			Rapid	0.0 Oct Mar	6.1 Dec	2.9
	0°C	7	Official	0.0 Nov	6.1 Oct	2.3
			Rapid	0.0 Oct	6.1 Dec	2.3
		14	Official	0.0 Nov Jan Apr	3.6 Dec	1.4
			Rapid	0.0 Oct	3.0 Sep Dec	1.7
	-20°C	10	Official	0.0 Nov Dec Jan Mar Apr	3.0 Sep Oct Feb	0.6
			Rapid	0.0 Nov Mar	3.6 Feb	1.4
		20	Official	0.0 Sep Oct Nov Dec Jan Feb Mar Apr	0.0	0.0
			Rapid	0.0 Sep Oct Nov Dec Jan Feb Mar Apr	0.0	0.0

Effect of Washing Treatment

The washing treatment reduced the overall mean coagulase-positive staphylococci values associated with iced oysters ($p < 0.01$) and frozen oysters ($p < 0.05$):

	Storage Temperature	
	0°C	-20°C
Unwashed Oysters	2.4	1.4
Washed Oysters	0.6	0.4

Effect (Reliability) of Laboratory Methodology

Both laboratory methodologies used, rapid and official, demonstrated close agreement on the overall numerical mean coagulase-positive staphylococci values associated with iced and frozen oysters. For iced oysters the mean value was 1.5 for both the official and rapid methodology, whereas for frozen oysters the mean value was 0.9 for the official and rapid methodology. Thus, there was no significant difference between the two methods.

Effect of Elapsed Storage Time

Increasing the length of time the oysters were held in cold storage greatly affected ($p < 0.01$) the mean numbers of coagulase-positive staphylococci in frozen oysters, whether washed or unwashed. The mean value for iced and frozen oysters decreased throughout the storage periods. The mean numerical coagulase-positive staphylococci values were:

Iced Oysters (0°C) Storage Period (Days)	Treatment Washed	Unwashed
0 (Initial)	0.9	3.4
7	0.5	2.3
14	0.3	1.6
Frozen Oysters (-20°C) Storage Period (Days)		
0 (Initial)	0.9	3.4
10	0.3	0.9
20	0.0	0.0

Effect of Harvest Month

The month in which the oysters were harvested had a very significant effect ($p < 0.01$) on the numbers of coagulase-positive staphylococci present in both iced and frozen oysters. The smallest and largest mean monthly values were associated with oysters collected in November and December, respectively, regardless of the temperature at which they were stored. The mean monthly coagulase-positive staphylococci values were:

Month	Iced Oysters (0°C)	Frozen Oysters (-20°C)
September	2.3	1.3
October	0.9	0.8
November	0.4	0.1
December	3.0	1.6
January	1.4	0.9
February	1.6	1.3
March	1.3	0.5
April	1.0	0.8

Effect of Interaction, Washing Treatment x Laboratory Methodology

For iced oysters stored at 0°C and frozen oysters stored at -20°C, the bivariate interaction, washing treatment x laboratory methodology, was not statistically significant. The mean coagulase-positive staphylococci values were;

Iced Oysters (0°C)	Laboratory Methodology	
	Official	Rapid
Washed	0.4	0.7
Unwashed	2.6	2.3
Frozen Oysters (-20°C)		
Washed	0.3	0.5
Unwashed	1.5	1.4

The results of the two laboratory methods were in close agreement. Washed oysters that had been stored at -20°C were associated with the smallest values; next were the values for washed oysters that had been stored at 0°C; then were the values for unwashed oysters that had been stored at -20°C and the largest values were associated with unwashed oysters that had been stored at 0°C. Thus, the relationship between washing treatment and laboratory methodology was consistent for all groups of oysters.

In Tables 31 - 40 (Appendix) are listed the detailed results of the analysis of variance of coagulase-positive staphylococci values of iced and frozen oysters, together with means for each of the four variables and their interactions.

Practical Application of the Rapid Coagulase-Positive Staphylococci Technique

The rapid procedure for enumeration of coagulase-positive staphylococci cannot entirely replace the official method. The latter has additional steps which act as limiting factors for inherent errors in the procedure. These include secondary MPN procedures on differential media and confirmatory MPN procedures in coagulase plasma.

SUMMARY AND CONCLUSIONS

Oysters deteriorate rapidly; therefore, it is imperative to determine their microbiological quality within a short period of time prior to processing and packing for commercial ditribution. Methods have been developed for assessing the bacterial quality of fresh shucked oysters which require a maximum of 24 hours. The reliability of these rapid methods, as demonstrated in this investigation, supports their implementation in the food industry. Their use could prevent considerable financial losses by eliminating unacceptable products prior to processing and packing.

Rapid methods developed for the enumeration of aerobic plate count (APC), total coliforms, E. coli and coagulase-positive Staphylococcus aureus, were used in this investigation to assess the bacterial quality of oysters. The rapid analytical procedures for determining the microorganisms were as follows: the APC procedure was a 24-hour plate count technique using Eugon agar; the procedures for total coliforms and E. coli were 24-hour LST MPNs; and the procedure for coagulase-positive staphylococci was a 24-hour MPN in TMM broth.

Samples of freshly harvested oysters grown in Barataria Bay were obtained monthly under standardized conditions from professional shuckers in New Orleans. The primary objective of this investigation was to determine the dependability of two different microbiological procedures, rapid and official for

estimating the numbers of organisms conventionally classified as APC, total coliforms, E. coli and coagulase-positive staphylococci in oysters. The effects of the following major sources of variation on the bacterial quality of oysters were also determined:

(a) two washing treatments which yielded washed and unwashed (salty) samples of oysters,

(b) two levels of temperature, 0°C and -20°C, at which the oysters were stored,

(c) three levels of elapsed time during which the oysters were held in cold storage, 0 days (receipt of oysters), 7 and 14 days for samples stored at 0°C, and 0, 10 and 20 days for samples stored at -20°C, and

(d) 8 monthly collections, September, 1975 - April, 1976.

Reliability of Rapid Methods

The results of the analysis of variance indicated the following regarding the four categories of organisms conventionally classified as APC, total coliforms, E. coli and coagulase-positive staphylococci found in the oysters:

1) No significant difference was found between the rapid and official techniques for the APC associated with oysters analyzed throughout iced storage.

2) In addition, no significant difference was found between the rapid and official techniques for total coliforms associated with oysters analyzed throughout frozen storage.

3) Also, no significant differences were found between the rapid and official techniques for E. coli or for coagulase-positive staphylococci associated with oysters, analyzed throughout iced and frozen storages.

Effect of Washing Treatment

Significant differences were found between the two washing treatments for each of the four categories of microorganisms.

Effect of Elapsed Storage Times

Oysters were bacteriologically examined immediately upon receipt and at various times during cold storage. Samples stored on ice (0°C) were analyzed after 7 and 14 days while samples stored at -20°C were analyzed after 10 and 20 days.

The results of the analysis of variance indicated that significant difference were found between the various elapsed storage times for the four categories of organisms conventionally classified as APC, total coliforms, E. coli and coagulase-positive staphylococci.

Effect of Harvest Months

Oysters were collected monthly from September, 1975 through April, 1976. Significant differences were found between the harvest months for each of the four categories of microorganisms.

Effect of Interaction Washing Treatment x Laboratory Methodology

The results of the analysis of variance indicated that no significant differences were found for the bivariate interaction washing treatment x laboratory methodology for each of the four categories of microoganisms,APC, total coliforms, E. coli and coagulase-positive staphylococci.

The above findings pertaining to each of the four categories of microorganisms indicate that the rapid methodologies, which require only 24 hours to perform, are capable of yielding reliable data for use in assessing the bacterial quality of oysters.

BIBLIOGRAPHY

American Public Health Association. 1910. Standard Methods for the Bacteriological Examination of Milk. American Public Health Association, New York.

American Public Health Association. 1965. Standard Methods for the Examination of Water and Wastewater. 12th ed. American Public Health Association, New York.

American Public Health Association. 1966. Recommended Methods for the Microbiological Examination of Foods. 2nd ed. American Public Health Association, New York.

American Public Health Association. 1967. Standard Methods for the Examination of Dairy Products. 12th ed. American Public Health Association, New York.

American Public Health Association. 1970. Control of Communicable Diseases in Man. 11th ed. American Public Health Association, New York.

American Public Health Association. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. 4th ed. American Public Health Association, New York.

Arcisz, W. 1951. Seasonal vairation of coliform and enterococci in a closed area. National Shellfisheries Association Proceedings.

Association of Official Analytical Chemists. 1970. Official Methods of Analysis of the Association of Official Analytical Chemists. 11th ed. Washington, D. C.

Baird-Parker, A.C. 1962. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. 25:12.

Barr, A. J. and J. H. Goodnight. 1971. Statistical Analysis System. Student Supply Store. N. C. State Univ., Raleigh.

Bergdoll, M. S., K. F. Weiss and M. J. Muster. 1967. The production of staphylococcal enterotoxin by a coagulase-negative organism. Bacteriol. Proc. 1967:12.

Berry, F. 1916. The bacterial content of market oysters. J. Bacteriol. 1:107.

Bicknell, A. K., F. Butt and L. Mattmen. 1952. Rapid identification of enteric organisms by microtechnique. Am. J. Public Health 25:437.

Brandish, J. M. and A. T. Willis. 1970. Observations on the coagulase and deoxyribonuclease tests for Staphylococcus. J. Med. Lab. Technol. 27:355.

Breed, R. S., E. G. Murray and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Md.

Burton, N. C. 1949. Comparison of coliform and enterococci organisms in frozen foods. Food Res. 14:434.

Butterfield, C. T. 1932. The selection of a dilution water for bacteriological examinations. J. Bacteriol. 23:355.

Chapman, G. H. 1944. A suggestion of the rapid presumptive examination of foods suspected of having caused staphylococcal food poisoning. Food Res. 9:377.

Chesbro, W. R. and K. Auborn. 1967. Enzymatic detection of the growth of the Staphylococcus aureus in foods. Appl. Microbiol. 15:1150.

Clark, H. F. and P. W. Kabler. 1964. Re-evaluation of the significance of the coliform bacteria. J. Am. Water Works Assoc. 56:931.

Cockran, W. C. and G. W. Snedecor. 1967. Statistical Methods. Iowa State University Press. Amer, Iowa.

Cobelli, V. and W. P. Hefferman. 1970. Seasonal factors relating to coliform levels in Northern quarham. Nat. Shellfisheries Assoc. Proc.

Corlett, D. A., Jr. 1974. Setting microbiological limits in the food industry. Food Technol. 28:34.

Dack, G. M., W. E. Cary, O. Woolpert and H. J. Wiggins. 1930. An outbreak of food poisoning proved to be due to a yellow hemolytic Staphylococcus. J. Prev. Med. 4:167.

Davis, J. G. 1939. A rapid, simple and reproducible method for the determination of bacterial sugar fermentations. Zenter. Bacteriol. Parasitenk., Abst. II, 101:97.

Davis, J. G. 1969a. Microbial standards for foods. Part I. Lab. Practise. 18:749.

Davis, J. G. 1969b. Microbial standard for foods. Part II. Lab. Practise. 18:839.

Davis, J. G. 1971. The use of alternate dilutions in making colony counts. Lab Practise. 20:871.

Difco Laboratories. 1953. Difco Manual. 9th ed. Difco Laboratories, Detroit, Michigan. 350p.

Dolman, C. E. and R. J. Wilson. 1938. Experiments with staphylococcal enterotoxin. J. Immunol. 35:13.

Eijkman, C. 1904. Die Garungaprobe bei 46°C als hilfsmittel bei de trinkwasseruntersuchung. Centr. Backteriol. Parasitenk., Abst. I, 37:742.

Escherich, T. 1885. Die darmbakterion des Hougeboronoo und Sauglings. Fortschr. d. Med. 3:515.

Eyre, J. W. H. 1924. The oyster and the public health. Am. J. Public Health. 38:6.

Evans, J. B. and C. F. Niven. 1950. A comparative study of known food poisoning staphylococci and related varieties. J. Bacteriol. 59:545.

Feldman, H. A. 1946. Incidence of certain biological characteristics among food poisoning staphylococci. Am. J. Public Health. 36:55.

Fishbein, M. 1962. The aerogenic response of Escherichia coli and strains of Aerobactor in EC broth and selected sugar broths at elevated temperatures. Appl. Microbiol. 10:79.

Fishbein, M., B. F. Surkeiwicz, E. F. Brown, H. M. Oxley, A. R. Padron and R. J. Grooms. 1967. Coliform behavior in frozen foods. 1. Rapid test for recovery of Escherichia coli from frozen foods. Appl. Microbiol. 15:233.

Fisher, L. M. and J. E. Acker. 1935. Baceriological examinations of oysters and water from Narragansett Bay. Sewage Works J. 7:727.

Foster, E. M., R. H. Deibel, C. L. Duncan, J. M. Goepfert and H. Sugiyama. 1970. Food Poisoning, p. 634 In Blair, J. E., E. H. Lennette and J. P. Truant. Manual of Clinical Microbiology. Am. Sec. for Microbiol., Bethesda, Md.

Frost, W. D. 1912. A rapid method for counting live bacteria in milk and other richly seeded materials. J. Am. Med. Assoc. 66:889.

Frost, W. D. 1921. Improved technique for the micro or little plate method in the routine colony count of milk samples. Am. J. Public Health 32:478.

Fugate, K. J., D. O. Oliver and M. T. Hatch. 1975. Enteroviruses and potential bacterial indicators in Gulf Coast oysters. J. Milk Food Technol. 38:100.

Fuller, C. A. 1902. Oysters and sewage in Narragansett Bay. Sci. 15:363.

Galton, M. M., J. E. Seatterday and A. V. Hardy. 1952. Bacterial epidemiology and clinical considerations of salmonellosis in dogs. J. Inf. Dis. 91:1.

Gage, S. M. 1910. Methods for testing shellfish for pollution. J. Inf. Dis. 7:78.

Geer, L. P., W. T. Murray and E. Smith. 1932. Bacterial content of frosted hamburger steak. Am. J. Public Health. 23:673.

Gorham, F. P. and S. Tonney. 1912. Seasonal variation in the bacterial content of oysters. Am. J. Public Health. 2:24.

Graunbaum, A. S. and E. H. Hume. 1902. Note on media for distinguishing Bacillus coli, Bacillus typhosa and related species. Brit. Med. J. 2:1473.

Griffin, A. M. and C. A. Stuart. 1940. An ecological study of coliform bacteria. J. Bacteriol. 40:83.

Gwatkin, R. 1937. Further observations of staphylococci infections of the bovine udder. Can. J. Public Health. 28:185.

Hanja, A. A. and C. A. Perry. 1935. A comparison of Eijkman Test with other tests for determining Escherichia coli. J. Bacteriol. 30:749.

Hammer, B. W. 1948. Dairy Bacteriology. 3rd ed. John Wiley and Sons, New York.

Hatfield, H. M. and W. H. Park. 1922. A study of the practical value of the Frost Little Plate Method in the routine colony count of milk and other samples. Am. J. Public Health. 32:478.

Hoff, J. C., W. J. Beck, T. H. Ericksen, G. J. Vasconcelos and M. W. Presnell. 1967. Time-temperature effect on the bacteriological quality of stored shellfish. J. Food Sci. 32:121.

Hoskins, J. K. 1933. The most probable number of Escherichia coli in water analysis. J. Am. Water Works Assoc. 25:867.

Hoskins, J. K. 1934. Most probable number for the evaluation of coliform-aerogenes tests by fermentation tube method. Public Health Rep. 49:393.

Houston, A. C. 1904. The bacteriological examination of oysters and estuarial waters. J. Hyg. 4:173.

Hunter, A. C. 1934. Need for methods for the bacteriological examination of crustacea. Am. J. Public Health. 24:199.

Hunter, A. C. 1939. Uses and limitations of the coliform group in sanitary control of food production. Food Res. 4:531.

Hunter, A. C. and C. W. Harrison. 1928 Bacteriology and chemistry of oysters. Bacterial flora of unpolluted oysters. Bacterial flora of polluted oysters. U. S. Dept. of Agr. Tech. Bull. No. 64.

Ingraham, J. L. 1958. Growth of psychrophilic bacteria, J. Bacteriol. 76:75.

Jennison, M. W. 1937. Relations between plate count and direct microscopic count of Escherichia coli during the logarithmic growth period. J. Bacteriol. 33:462.

Jennison, M. W. and E. Wadsworth. 1940. Evaluation of the errors involved in estimating bacterial numbers by the plating method. J. Bacteriol. 36:389.

Johnstone. 1909. Routine methods of shellfish examination with reference to sewage pollution. J. Hyg. 9:412.

Jordan, E. C. 1931. Staphylococcus food poisoning. J. Am. Med. Assoc. 94:1648.

Joshi, N. and D. C. Dale. 1963. Some microbiological aspects of staphylococcal mastitis. Can. J. Comp. Med. 27:61.

Kachikian, R., C. R. Fellers and W. Litsky. 1959. A bacterial survey of commercial frozen breaded shrimp. J. Milk Food Technol. 22:310.

Kelly, C. B. 1960. Bacteriological criteria for market oysters. Procedures for the bacteriological examination of oysters. U. S. Department of Health, Education, and Welfare. Robert A. Taft Sanitary Engineering Center. Tech. Rep. No. F60-2.

Kelly, C. B., M. W. Presnell and K. J. ZoBell. 1961. Effect of commercial handling and storage on the bacteriological quality of Pacific oysters. p. 203. In Proc. of the Shellfish Sanitation Workshop. U. S. Dept. of Health, Education and Welfare. Washington, D. C.

Kendall, J. H. 1973. New procedures for the rapid detection of coliforms and Escherichia coli in breaded shrimp. PhD dissertation, Louisiana State University, Baton Rouge.

Klein, D. A. and W. Shenyuh. 1974. Stress: a factor to be considered in heterotrophic microorganisms enumeration from acquatic environments. Appl. Microbiol. 27:429.

Klein, E. 1896. Supp. 24th. Annual Report of the Local Gov't. Bd. 1894-1896. Oyster culture in relation to disease: a continuation of the report of the Medical Officer for 1894-1895. 37:109.

Lachica, R. V. F., P. D. Hoeprich and C. Genigeogis. 1971. Nuclease production and lysostaphin susceptibility of Staphylococcus aureus and other catalase-positive cocci. Appl. Microbiol. 21:823.

Lachica, R. V. F., V. K. Weiss and R. H. Deibel. 1969. Relationship among coagulase, enterotoxin and heat-stable deoxyribonuclease production in Staphylococcus aureus. Appl. Microbiol. 18:126.

Levine, G. V., V. R. Harrison and W. C. Hess. 1957. Use of radioactive culture media. J. Am. Water Works Assoc. 9:1069.

Liebman, H. L., G. Kudo, J. G. Chapel and J. A. Stern. 1957. Effects of temperature upon the storage life of fresh shucked Pacific oysters (Ostrea gigas). Commercial Fisheries Rev. 19: No. 12. Washington, D. C.

Lofton, C. B., S. M. Morrison and P. D. Leiby. 1962. The enterobacteriaceae of some Colorado small mammals and birds and their possible role in gastroenteritis in man and domestic animals. Z. Res. 1:277.

Lord, J. 1941. Sanitary quality of market oysters. PhD dissertation, Univ. of Illinois, Urbana.

McDale, J. J. and R. A. Weaver. 1959. Rapid methods for the detection of carbohydrate fermentation. J. Bacteriol. 77:65.

Miller, A. P. 1937. Waste disposal as related to shellfish. Sewage Works J. 9:482.

Minor, T. E. and E. H. Marth. 1971. Staphylococcus aureus and staphylococcal food intoxications. A review. I. The staphylococci: characteristics, isolation and behavior in artificial media. J. Milk Food Technol. 34:557.

Moldovan, A. A. 1935. A modified technique for the detection of the Escherichia coli - Aerobacter group in milk. Am. J. Public Health. 25:1035.

Moldovan, A. A. 1965. A simplified coliform test for milk products. J. Milk Food Technol. 28:220.

Mudge, C. S. and B. M. Lawler. 1928. Is the statistical method applicable to the bacterial plate count? J. Bacteriol. 15:207.

Murchelano, R. A., C. Brown and J. Bishop. 1975. Quantitative and qualitative studies of bacteria isolated from sea water used in the laboratory culture of the American oyster, Crassostrea virginica. J. Fisheries Res. Bd. Can. 32:739.

Nelson, F. E. and M. P. Barder. 1954. The influence of time and temperature of plate incubation upon bacterial counts on market milk and related products, particularly after holding under refrigeration. J. Milk Food Technol. 17:95.

Nickerson, J. T. and A. J. Sinksey. 1972. Microbiology of foods and food processing. Am. Elsevier Pub. Co., New York.

Nottingham, P. M. and A. J. Urselmann. 1961. Salmonella infection in calves and other animals. New Zealand J. Agr. Res. 4:449.

Old, H. N. 1932. Oyster sanitation. Proc. of the 9th Annual Short School. Texas Public Health Assoc. p. 65.

Olsen, J. C., Jr. 1968. Microbiological considerations in light of today's food processing operations. J. Milk Food Technol. 31:335.

Paponnet, A. and J. Brisou. 1937a. "Fruits de mer" et salmonellosis dans la region du lack et Bigerte. A Arch. Inst. Pasteur de Tunis. 26:676.

Paponnet, A. and J. Brisou. 1937b. Rescherches bacteriologiques sur les coquillages. Etudes de quelques salmonelles. Considerations generales. Rev. d' Hyg. et de Med. Preventive. 59:466.

Parr, L. W. 1939. Coliform bacteria. Bacteriol. Rev. 3:1.

Parr, L. W. and F. Kaufmann. 1951. Enterobacteriaceae. Munksgard Enar Pub. Co., Copenhagen.

Perry, C. A. 1939. A summary of studies of pollution in shellfish. Food Res. 4:381.

Perry, C. A. and M. Bayliss. 1936. Escherichia coli as an indicator of fecal pollution in oysters, crabmeat and other substances. Am. J. Public Health. 26:406.

Pottinger, S. R. 1944. Preparation of Eastern oysters for market. U. S. Dept. of Interior. Bureau of Fisheries. Leaflet No. 50. Chicago, Illinois.

Prescott, S. C., C. Winslow, A. Winslow and M. H. McGrady. 1946. Water Bacteriology. 6th ed. John Wiley and Sons, Inc. New York.

Presnell, M. W. and C. B. Kelly. 1961. Bacteriological studies of commercial shellfish operations on the Gulf Coast. I. Observations in oyster growing areas. IIA. Changes during harvesting, processing and storage of oysters. IIB. Evaluation of methods for enumerating Escherichia coli. U. S. Dept. of Health, Education and Welfare. Public Health Service. Robert A. Taft Sanitary Engineering Center. Technical Rep. No. F619.

Quist, K. D. 1962. Salmonella in poultry as related to human health. U. S. Dept. of Agr., Rep. of National Plans Conference.

Rottini, G. D. and T. Zacchi. 1968. Escherichia coli as a cause of gastroenteritis. J. Microbiol. 16:189.

Round, L. A. 1916. Comparative bacteriological examination of shell liquor and meats of oysters. Am. J. Public Health. 6:686.

Schacht, T. L. and A. H. Robertson. 1932. Accuracy of counting an agar plate. Am. J. Public Health. 22:80.

Sharpe, A. N., E. J. Dyett, A. K. Jackson and D. C. Kilsby. 1972. Technique and apparatus for rapid and inexpensive enumeration of bacteria. Appl. Microbiol. 22:531.

Sharpe, A. N., and D. C. Kilsby. 1972. A rapid, inexpensive bacterial count technique using agar droplets. J. Appl. Bacteriol. 34:435.

Sherman, J. M. and H. U. Wing. 1933. The significance of bacteria in milk with special reference to standards. J. Dairy Sci. 16:165.

Stiles, C. W., Jr. 1911. The bacteriological examination of shucked and shell oysters. J. Am. Public Health 1:623.

Stuart, R. Q. and R. H. Weaver. 1948. A rapid method for confirmatory coliform analysis. J. Bacteriol. 56:53.

Summers, J. L. 1967. The sanitary significance of pollution of waters by domestic and wild animals. U. S. Dept. of Health, Education and Welfare. Public Health Service. Shellfish Sanitation Technical Report.

Tanon, L. and R. Neveu. 1932. Etude sur 1'etat sanitaire des huitres vendues a Paris en 1931-1932. Bull. Acad. de Med. Paris. 107:435.

Tennant, A. D. 1955. Bacteriological indices of pollution in oyster areas in Prince Edward Island. PhD dissertation, McGill University, Montreal, Quebec, Canada.

Thatcher, F. S. and D. S. Clark. 1968. Microorganisms in foods: their significance and methods of enumeration. University of Toronto Press, Toronto.

Thatcher, F. S. and W. A. Simmons. 1956. Comparative appraisal of the properties of staphylococci isolated from clinical sites and from dairy products. Can. J. Microbiol. 2:703.

Thomas, S. B. 1955. Coli-aerogenes bacteria in raw milk. J. Appl. Bacteriol. 18:331.

Tonney, F. O. and J. L. White. 1926. Viability of Bacillus typhosus in oysters during storage. J. Am. Med. Assoc. 84:1403.

Tressler, D. 1937. The freezing preservation of oysters. Nat. Shellfisheries Assoc. Proc.

Truitt, R. V. 1929. Biological contributions to the development of the oyster industry in Maryland. American University Press, Washington, D. C.

Turck, M. and R. C. Petersdorf. 1962. The epidemiology of nonenteric Escherichia coli infection; prevalence of serological groups. J. Clin. Invest. 41:1760.

Turck, M., R. H. Winterbauer, E. Atlas, F. Silverblott and R. G. Petersdorf. 1969. Studies on the epidemiology of Escherichia coli. J. Inf Dis. 120:13.

U. S. Department of Health, Education and Welfare. 1962. Recommended procedures for the bacteriological examination of sea water and shellfish. 3rd ed. U. S. Dept. of Health, Education and Welfare. Public Health Service, New York.

U. S. Department of Health, Education and Welfare. 1968. Bacteriological Analytical Manual for Foods. 2nd ed. U. S. Dept. of Health, Education and Welfare, Public Health Service, Consumer Protection and Environmental Health Service. Food and Drug Administration, Division of Microbiol. Washington, D. C.

U. S. Department of Health, Education and Welfare. 1969. Bacteriological Analytical Manual for Foods. 2nd ed. U. S. Dept. of Health, Education and Welfare, Public Health Service; Consumer Protection and Environmental Health Service. Food and Drug Administration, Division of Microbiol. Washington, D. C.

U. S. Department of Health, Education and Welfare. 1965. National Shellfish Sanitation Program Manual of Operations. I. Sanitation of Shellfish Growing Areas. II. Sanitation of the Harvesting and Processing of Shellfish. III. Public Health Service Appraisal of State Shellfish Sanitation Programs. U. S. Dept. of Health, Education and Welfare, Public Health Service, Division of Environmental Engineering and Food Protection, Shellfish Sanitation Branch. Washington, D. C.

Vanderzant, C., C. A. Thompson, Jr. and S. M. Ray. 1973. Microbial flora and level of Vibrio parahemolyticus of oysters (Crassostrea virginica), water and sediment from Galveston Bay. J. Milk Food Technol. 36:9.

Vanderzant, C. and A. Y. Moore. 1955. Some factors affecting bacterial counts of soft ice-milk. Food Technol. 9:285.

Vaughn, V. C. 1884. Poisonous sick cheese. Public Health Rep. 10:241.

Weinziel, J. and E. B. Newton. 1914. Bacteriological methods for meat analysis. Am. J. Public Health 4:408.

White, J. 1926. Bacillus coli in market oysters. Am. J. Public Health. 16:597.

Wilson, E., M. J. Foster and K. H. Lewis. 1959. A rapid test for detecting Staphylococcus aureus in food. Appl. Microbiol. 7:22.

Wilson, T. E. and C. S. McCleskey. 1951. The effect of storage on indicator organisms in shucked oysters and a comparison of methods for the determination of enterococci. Food Res. 16:377.

Winter, F. H., G. K. York and H. E. Nakhai. 1971. Quick counting method for estimating the number of viable microbes on food and food processing equipment. Appl. Microbiol. 22:510.

Wise, R. L., J. B. Winston and C. Culli, Jr. 1948. Factors of sewage pollution in oyster beds in Galveston Bay. Am. J. Public Health. 38:1109.

Youden, W. J. 1951. Statistical Methods for Chemists. John Wiley and Sons, New York.

Zemelman, R. and L. Longeri. 1965. Characterization of staphylococci isolated from raw milk. Appl. Microbiol. 13:167.

ZoBell, C. E. and J. E. Conn. 1940. Studies on thermal sensitivity of marine bacteria. J. Bacteriol. 40:223.

APPENDIX

Table A Composition of Plate Count Agar

Dextrose	1 g
Yeast Extract	3 g
Tryptose	5 g
Agar	15 g
Distilled water	1000 ml

Table B Composition of Eugon Agar

L-Cystine	0.2 g
Sodium sulfite	0.2 g
Sodium citrate	1 g
Sodium chloride	4 g
Soytone	5 g
Dextrose	5 g
Agar	15 g
Tryptose	15 g
Distilled water	1000 ml

Table C Composition of Lauryl Sulfate Tryptose Broth

Sodium lauryl sulfate	0.1 g
Dipotassium phosphate	2.75 g
Monopotassium phosphate	2.75 g
Lactose	5.0 g
Sodium chloride	5.0 g
Tryptose	20.0 g
Distilled water	1000.0 ml

Table D Composition of EC Medium

Bile salts No. 3	1.5 g
Monopotassium phosphate	1.5 g
Dipotassium phosphate	4.0 g
Sodium chloride	5.0 g
Lactose	5.0 g
Tryptose	20.0 g
Distilled water	1000.0 ml

Table E Composition of Brillian Green Lactose Bile Broth (2%)

Brillian green	0.0133	g
Peptone	10.0	g
Lactose	10.0	g
Oxgall	20.0	g
Distilled water	1000.0	ml

Table F Composition of Levine EMB Agar

Methylene blue	0.065	g
Eosin Y	0.4	g
Dipotassium phosphate	2.0	g
Peptone	10.0	g
Lactose	10.0	g
Agar	15.0	g
Distilled water	1000.0	ml

Table G Composition of Trypticase Soy Broth

Dipotassium phosphate	2.5 g
Soytone	3.0 g
Dextrose	5.0 g
Tryptone	17.0 g
Sodium chloride	100.0 g
Distilled water	1000.0 ml

Table H Composition of Vogel-Johnson Agar

Phenol red	0.25 g
Yeast extract	5.0 g
Dipotassium phosphate	5.0 g
Lithium chloride	5.0 g
Tryptone	10.0 g
Mannitol	10.0 g
Glycine	10.0 g
Agar	15.0 g
Distilled Water	1000.0 ml

Table I Composition of Brain Heart Infusion Broth

Dextrose	2.0 g
Disodium phosphate	2.5 g
Sodium chloride	5.0 g
Proteose peptone	10.0 g
Infusion from calf brains	250.0 g
Distilled water	1000.0 ml

Table J Composition of Tryptic Mannitol Meat Broth

Phenol red	0.12 g
d- Mannitol	2.5 g
Dipotassium phosphate	2.5 g
Soytone	3.0 g
Proteose peptone	4.5 g
Tryptone	17.0 g
Beef heart infusion	100.0 g
Sodium chloride	100.0 g
Distilled water	1000.0 ml

Table 1 Analysis of Variance for Aerobic Plate Count Values of Oysters Stored at 0°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	418,750,184	658.62**
C	2	2,923,518,761	4,598.18**
D	7	60,170,889	94.64**
AC	2	300,883,707	473.24**
AD	7	53,562,304	84.24**
CD	14	51,297,498	80.68**
ACD	14	46,360,118	72.92**
Error Term for Main Plots: Dup (A C D)	48	635,800	
Subplots			
B	1	6,314	0.0012
AB	1	61,884	0.0118
BC	2	25,597	0.0049
BD	7	2,797,200	0.5340
ABC	2	63,404	0.0121
ABD	7	2,354,679	0.4495
BCD	14	2,393,709	0.4570
ABCD	14	2,028.110	0.3872
Residual	48	5,237,980	

**$p < 0.01$

Table 2 Mean Aerobic Plate Count Values of Oysters Stored at 0°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Quadrivariate Interaction, A x B x C x D.						Trivariate, A x C x D	
C. Day	D. Mo.	Washed Official	Washed Rapid	Unwashed Official	Unwashed Rapid	Washed	Unwashed
0	1	99.0	110.0	415.0	412.5	104.5	413.8
	2	83.0	78.0	296.5	357.5	80.5	327.0
	3	86.0	79.0	340.0	320.0	82.5	330.0
	4	88.0	71.0	315.0	310.0	79.5	312.5
	5	57.5	56.0	201.5	216.0	56.8	208.8
	6	86.0	71.5	252.0	223.5	78.8	237.8
	7	92.5	86.0	377.5	305.0	89.2	341.2
	8	109.0	73.5	357.5	395.0	91.2	376.2
7	1	85.5	85.5	450.0	507.5	85.5	478.8
	2	83.5	75.0	347.5	400.0	79.2	373.8
	3	216.0	205.0	1105.0	1025.0	210.5	1065.0
	4	234.0	169.0	1315.0	1240.0	201.5	1277.5
	5	129.0	133.0	452.5	645.0	131.0	548.8
	6	207.5	226.5	560.0	770.0	217.0	665.0
	7	189.0	180.5	1922.5	1395.0	184.8	1658.8
	8	94.0	63.5	487.5	485.0	78.8	486.2
14	1	9550.0	10250.0	10750.0	10750.0	9900.0	10750.0
	2	8130.0	7725.0	9575.0	10225.0	7927.5	9900.0
	3	7750.0	7925.0	20400.0	20250.0	7837.5	20325.0
	4	7425.0	6550.0	23350.0	22400.0	6987.5	22875.0
	5	5000.0	5125.0	8800.0	8425.0	5062.5	8612.5
	6	8275.0	8450.0	7450.0	15300.0	8362.5	11375.0
	7	9250.0	8875.0	36000.0	30250.0	9062.5	33125.0
	8	9375.0	9445.0	11200.0	11250.0	9410.0	11225.0
		F value for ABCD = 0.387 (d.f. for ABCD = 14/48)				F value for ACD = 72.92** (d.f. for ACD = 14/48)	

**p < 0.01

Table 3 Mean Aerobic Plate Count Values of Oysters Stored at 0°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	257.0	261.2	259.1
	2	189.8	217.8	203.8
	3	213.0	199.5	206.2
	4	201.5	190.5	196.0
	5	129.5	136.0	132.8
	6	169.0	147.5	158.2
	7	235.0	195.5	215.2
	8	233.2	234.2	233.8
7	1	267.8	296.5	282.1
	2	215.5	237.5	226.5
	3	660.5	615.0	637.8
	4	774.5	704.5	739.5
	5	290.8	389.0	339.9
	6	383.8	498.2	441.0
	7	1055.8	787.8	921.8
	8	290.8	274.2	282.5
14	1	10150.0	10500.0	10325.0
	2	8852.5	8975.0	8913.8
	3	14075.0	14087.5	14081.2
	4	15387.5	14475.0	14931.2
	5	6900.0	6775.0	6837.5
	6	7862.5	11875.0	9868.8
	7	22625.0	19562.5	21093.8
	8	10287.5	10347.5	10317.5
		F value for BCD = 0.457 (d.f. for BCD = 14/48)		F value for CD = 80.68** (d.f. for CD = 14/48)

**p < 0.01

Table 4 Mean Aerobic Plate Count Values of Oysters Stored at 0°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	3244.8	3481.8	3871.7	3890.0
2	October	2765.5	2626.0	3406.3	3660.8
3	November	2684.0	2736.3	7281.7	7198.3
4	December	2582.3	2263.3	8326.7	7983.3
5	January	1728.8	1771.3	3151.3	3095.3
6	February	2856.2	2916.0	2754.0	5431.2
7	March	3177.2	3047.2	12766.7	10650.0
8	April	3192.7	3194.0	4015.0	4043.3

F value for ABD = 0.450
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment Washed	A. Treatment Unwashed
1	3363.3	3880.8
2	2695.8	3533.6
3	2710.2	7240.0
4	2422.8	8155.0
5	1750.1	3123.3
6	2886.1	4092.6
7	3112.2	11708.3
8	3193.3	4029.2

F value for AD = 84.24**
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method Official	B. Method Rapid
3558.2	3685.9
3085.9	3143.4
4982.8	4967.3
5454.5	5123.3
2440.1	2433.3
2805.1	4173.6
7971.9	6848.6
3603.8	3618.7

F value for BD = 0.534
(d.f. for BD = 7/48)

Harvest Month Means - D
3622.1
3114.7
4975.1
5288.9
2436.7
3489.3
7410.2
3611.2

F value for D = 94.64**
(d.f. for D = 7/48)

**p < 0.01

Table 5 Mean Aerobic Plate Count Values of Oysters Stored at 0°C for ABC, AB, AC, BC, A, B and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	7	14	
Washed	Official	87.6	154.8	8094.4	2778.9
	Rapid	78.1	142.2	8043.1	2754.5
Unwashed	Official	319.4	830.0	15940.6	5696.7
	Rapid	317.4	808.4	16106.2	5744.0
		F value for ABC = 0.012 (d.f. for ABC = 2/48)			F value for AB = 0.012 (d.f. for AB = 1/48)
Bivariate Interaction, A x C					A. Treatment Means
A. Treatment					
Washed		82.9	148.5	8068.8	2766.7
Unwashed		318.4	819.2	16023.4	5720.4
		F value for AC = 473.24** (d.f. for AC = 2/48)			F value for A = 658.62** (d.f. for A = 1/48)
Bivariate Interaction, B x C					B. Method Means
B. Method					
Official		203.5	492.4	12017.5	4237.8
Rapid		197.8	475.3	12074.7	4249.3
		F value for BC = 0.005 (d.f. for BC = 2/48)			F value for B = 0.0012 (d.f. for B = 1/48)
C. Elapsed Time Means		200.6	483.9	12046.1	Overall Mean 4243.5
		F value for C = 4598.18** (d.f. for C = 2/48)			

**p < 0.01

Table 6 Analysis of Variance for Aerobic Plate Count Values of Oysters Stored at -20°C

Vairable: A = Washing Treatment; B = Laboratory Methodology: C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	1,830,078.26	1,296.60**
C	2	172,853.69	122.47**
D	7	34,200.77	24.23**
AC	2	49,433.15	35.02**
AD	7	19,482.49	13.80**
CD	14	3,754.50	2.66**
ACD	14	3,276.85	2.32*
Error Term for Main Plots			
Dup (A C D)	48	1,411.44	
Subplots			
B	1	1,068.80	5.8237*
AB	1	148.76	0.8105
BC	2	222.25	1.2110
BD	7	730.82	3.9821**
ABC	2	75.27	0.4101
ABD	7	1,152.45	6.2795**
BCD	14	405.17	2.2077*
ABCD	14	389.89	2.1244*
Residual	48	183.53	

**$p < 0.01$
*$p < 0.05$

Table 7 Mean Aerobic Plate Count Values of Oysters Stored at -20°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate, A x C x D	
C.	D.	Washed		Unwashed			
Day	Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	99.0	110.0	415.0	412.5	104.5	413.8
	2	83.0	78.0	296.5	357.5	80.5	327.0
	3	86.0	79.0	340.0	320.0	82.5	330.0
	4	88.0	71.0	315.0	310.0	79.5	312.5
	5	57.5	56.0	201.5	316.0	56.8	208.8
	6	86.0	71.5	252.0	223.5	78.8	237.8
	7	92.5	86.0	377.5	305.0	89.2	341.2
	8	109.0	73.5	357.5	395.0	91.2	376.2
10	1	55.0	53.0	244.5	264.5	54.0	254.5
	2	43.5	39.5	145.0	199.0	41.5	172.0
	3	33.5	36.5	153.0	161.0	34.8	157.0
	4	39.0	36.5	206.0	174.5	37.8	190.2
	5	22.5	21.5	110.5	100.0	22.0	105.2
	6	39.0	36.0	76.0	88.5	37.5	82.2
	7	37.0	39.0	194.0	165.5	38.0	179.8
	8	52.5	38.5	231.0	218.5	45.5	224.8
20	1	104.5	99.5	420.0	365.0	102.0	392.5
	2	77.0	67.5	378.0	367.5	72.2	372.8
	3	85.0	82.5	287.5	307.5	83.8	297.5
	4	69.0	69.0	345.0	325.0	69.0	335.0
	5	58.0	53.5	198.5	211.0	55.8	204.8
	6	77.5	71.0	154.0	153.5	74.2	153.8
	7	90.5	87.0	412.5	380.0	88.8	396.2
	8	94.5	67.5	212.0	231.0	81.0	221.5
		F value for ABCD = 2.124* (d.f. for ABCD = 14/48)				F value for ACD = 2.322* (d.f. for ACD = 14/48)	

*$p < 0.05$

Table 8 Mean Aerobic Plate Count Values of Oysters Stored at -20°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	257.0	261.2	259.1
	2	189.8	217.8	203.8
	3	213.0	199.5	206.2
	4	201.5	190.5	196.0
	5	129.5	136.0	132.8
	6	169.0	147.5	158.2
	7	235.0	195.5	215.2
	8	233.2	234.2	233.8
10	1	149.8	158.8	154.2
	2	94.2	119.2	106.8
	3	93.0	98.8	95.9
	4	122.5	105.5	114.0
	5	66.5	60.8	63.6
	6	57.5	62.2	59.9
	7	115.5	102.2	108.9
	8	141.8	128.5	135.1
20	1	262.2	232.2	247.2
	2	227.5	217.5	222.5
	3	186.2	195.0	190.6
	4	207.0	197.0	202.0
	5	128.2	132.2	130.2
	6	115.8	112.2	114.0
	7	251.5	233.5	242.5
	8	153.2	149.2	151.2
		F value for BCD = 2.208* (d.f. for BCD = 14/48)		F value for CD = 2.660** (d.f. for CD = 14/48)

*$p < 0.05$
**$p < 0.01$

Table 9 Mean Aerobic Plate Count Values of Oysters Stored at -20°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	86.2	87.5	359.8	347.3
2	October	67.8	61.7	273.2	308.0
3	November	68.0	66.0	260.2	262.8
4	December	65.3	58.8	288.7	269.8
5	January	46.0	43.7	170.2	175.7
6	February	67.5	59.5	160.7	155.2
7	March	73.3	70.7	328.0	283.5
8	April	85.3	59.8	266.8	281.5

F value for ABD = 6.279**
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment: Washed	A. Treatment: Unwashed
1	86.8	353.6
2	64.8	290.6
3	67.0	261.5
4	62.1	279.2
5	44.8	172.9
6	63.5	157.9
7	72.0	305.8
8	72.6	274.2

F value for AD = 13.803**
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method: Official	B. Method: Rapid
223.0	217.4
170.5	184.8
164.1	164.4
177.0	164.3
108.1	109.7
114.1	107.3
200.7	177.1
176.1	170.7

F value for BD = 3.892**
(d.f. for BD = 7/48)

Harvest Month Means - D
220.2
177.7
164.2
170.7
108.9
110.7
188.9
173.4

F value for D = 24.231**
(d.f. for D = 7/48)

**p < 0.01

Table 10 Mean Aerobic Plate Count Values of Oysters Stored at -20°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	10	20	
Washed	Official	87.6	40.2	82.0	69.9
	Rapid	78.1	37.6	74.7	63.5
Unwashed	Official	319.4	170.0	300.9	263.4
	Rapid	317.4	171.4	292.6	260.5
		F value for ABC = 0.410 (d.f. for ABC = 2/48)			F value for AB = 0.811 (d.f. for AB = 1/48)
Bivariate Interaction, A x C					A. Treatment Means
A. Treatment					
Washed		82.9	38.9	78.3	66.7
Unwashed		318.4	170.7	296.8	261.0
		F value for AC = 35.023** (d.f. for AC = 2/48)			F value for A = 1,296.60** (d.f. for A = 1/48)
Bivariate Interaction, B x C					B. Method Means
B. Method					
Official		203.5	105.1	191.5	166.7
Rapid		197.8	104.5	183.6	162.0
		F value for BC = 1.211 (d.f. for BC = 2/48)			F value for B = 5.824* (d.f. for b = 1/48)
C. Elapsed Time Means		200.6	104.8	187.5	Overall Mean 164.3
		F value for C = 122.466** (d.f. for C = 2/48)			

*p < 0.05
**p < 0.01

Table 11 Analysis of Variance for Total Coliform MPN Values of Oysters Stored at 0°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	123,728.52	39.53**
C	2	403,078.07	128.78**
D	7	96,525.64	30.84**
AC	2	77,465.97	24.75**
AD	7	39,552.69	12.64**
CD	14	66,198.92	21.15**
ACD	14	37,641.94	12.03**
Error Term for Main Plots: Dup (A C D)	48	3,129.969	
Subplots			
B	1	17,980.02	6.0496*
AB	1	10,472.52	3.5236
BC	2	9,536.76	3.2088*
BD	7	4,585,12	1.5427
ABC	2	8,197.60	2.7582
ABD	7	1,255,28	0.4224
BCD	14	3,022.55	1.0170
ABCD	14	982.70	0.3306
Residual	48	2,972.09	

**$p < 0.01$
*$p < 0.05$

Table 12 Mean Total Coliform MPN Values of Oysters Stored at 0°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate, A x C x D	
C.	D.	Washed		Unwashed			
Day	Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	94.0	84.0	107.5	85.0	89.0	96.3
	2	64.0	69.5	84.0	75.0	66.8	79.5
	3	42.0	48.5	48.5	53.0	45.3	50.8
	4	43.5	42.0	58.5	48.5	42.8	53.5
	5	59.0	41.0	43.0	41.5	40.0	42.3
	6	46.0	43.0	58.5	53.0	44.5	55.8
	7	69.5	58.5	75.0	64.0	64.0	69.5
	8	84.0	75.0	94.0	97.5	79.5	95.8
7	1	75.0	69.5	106.5	85.0	72.3	95.8
	2	59.5	58.5	84.0	64.0	59.0	74.0
	3	64.0	78.5	78.5	94.0	71.3	86.3
	4	64.0	48.0	69.5	53.0	56.3	61.3
	5	84.0	69.5	85.0	75.0	76.8	80.0
	6	58.5	53.0	69.5	58.5	55.8	64.0
	7	78.5	64.0	85.0	84.0	71.3	84.5
	8	64.0	69.5	79.5	84.0	66.8	81.8
14	1	265.0	185.0	1100.5	780.0	225.0	940.3
	2	200.0	185.0	335.0	210.0	192.5	272.5
	3	85.0	106.5	106.5	107.5	95.8	107.0
	4	79.5	84.0	92.0	85.0	81.8	88.5
	5	84.0	75.0	160.0	95.0	79.5	127.5
	6	69.5	69.5	85.0	84.0	69.5	84.5
	7	160.0	135.0	350.0	200.0	147.5	275.0
	8	200.0	250.0	290.0	250.0	225.0	270.0
		F value for ABCD = 0.3306 (d.f. for ABCD = 14/48)				F value for ACD = 12.03** (d.f. for ACD = 14/48	

**p < 0.01

Table 13 Mean Total Coliform MPN Values of Oysters Stored at 0°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	100.8	84.5	92.6
	2	74.0	72.3	73.1
	3	45.3	50.8	48.0
	4	51.0	45.3	48.1
	5	41.0	41.3	41.1
	6	52.3	48.0	50.1
	7	72.3	61.3	66.8
	8	89.0	86.3	87.6
7	1	90.8	77.3	84.0
	2	71.8	61.3	66.5
	3	71.3	86.3	78.8
	4	66.8	50.8	58.8
	5	84.5	72.3	78.4
	6	64.0	55.8	59.9
	7	81.8	74.0	77.9
	8	71.8	76.8	74.3
14	1	682.8	482.5	582.6
	2	267.5	197.5	232.5
	3	95.8	107.0	101.4
	4	85.8	84.5	85.1
	5	122.0	85.0	103.5
	6	77.3	76.8	77.0
	7	255.0	167.5	211.3
	8	245.0	250.0	247.5
		F value for BCD = 1.017* (d.f. for BCD = 14/48)		F value for CD = 21.150** (d.f. for CD = 14/48

*p < 0.05
**p < 0.01

Table 14 Mean Total Coliform MPN Values of Oysters Stored at 0°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	144.7	112.8	438.2	316.7
2	October	107.8	104.3	167.7	116.3
3	November	63.7	77.8	77.8	84.8
4	December	62.3	58.2	73.3	62.2
5	January	69.0	61.8	96.0	70.5
6	February	58.0	55.2	71.0	65.2
7	March	102.7	85.8	170.0	116.0
8	April	116.0	131.5	154.5	143.8

F value for ABD = 0.422
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment: Washed	A. Treatment: Unwashed
1	128.8	377.4
2	106.1	142.0
3	70.8	81.3
4	60.3	67.8
5	65.4	83.3
6	56.6	68.1
7	94.3	143.0
8	123.8	149.2

F value for AD = 12.637**
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method: Official	B. Method: Rapid
291.4	214.8
137.8	110.3
70.8	81.3
67.8	60.2
82.5	66.2
64.5	60.2
136.3	100.9
135.3	137.7

F value for BD = 1.543*
(d.f. for BD = 7/48)

Harvest Month Means - D
253.1
124.0
76.0
64.0
74.3
62.3
118.6
136.4

F value for D = 30.839**
(d.f. for D = 7/48)

**p < 0.01
*p < 0.05

Table 15 Mean Total Coliform MPN Values of Oysters Stored at 0°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	7	14	
Washed	Official	60.3	68.4	142.9	90.5
	Rapid	57.7	63.9	136.3	85.9
Unwashed	Official	71.2	82.2	314.9	156.1
	Rapid	64.7	74.7	226.4	121.9
		F value for ABC = 2.758* (d.f. for ABC = 2/48)			F value for AB = 3.524* (d.f. for AB = 1/48)
Bivariate Interaction, A x C					**A. Treatment Means**
A. Treatment					
Washed		59.0	66.2	139.6	88.2
Unwashed		67.9	78.4	270.7	139.0
		F value for AC = 24.750** (d.f. for AC = 2/48)			F value for A = 39.530** (d.f. for A = 1/48)
Bivariate Interaction, B x C					**B. Method Means**
B. Method					
Official		65.7	75.3	228.9	123.3
Rapid		61.2	69.3	181.3	103.9
		F value for BC = 3.208* (d.f. for BC = 2/48)			F value for B = 6.050* (d.f. for B = 1/48)
C. Elapsed Time Means		63.4	72.3	205.1	Overall Mean 113.6
		F value for C = 128.780** (d.f. for C = 2/48)			

**p < 0.01
*p < 0.05

Table 16 Analysis of Variance for Total Coliform MPN Values of Oysters Stored at -20°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	668.64	17.39**
C	2	79,560.60	2,069.11**
D	7	1,272.59	33.10**
AC	2	326.59	8.49**
AD	7	14.66	0.38
CD	14	918.98	23.90**
ACD	14	15.01	0.39
Error Term for Main Plots: Dup (ACD)	48	38.4516	
Subplots			
B	1	73.88	2.6295
AB	1	26.33	0.9434
BC	2	127.86	4.5816*
BD	7	33.78	1.2103
ABC	2	17.72	0.6351
ABD	7	12.08	0.4327
BCD	14	33.94	1.2160
ABCD	14	12.37	0.4431
Residual	48	27.91	

**p < 0.01
*p < 0.05

Table 17 Mean Total Coliform MPN Values of Oysters Stored at -20°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D - Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate. A x C x D	
		Washed		Unwashed			
C. Day	D. Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	94.0	84.0	107.5	85.0	89.0	96.3
	2	64.0	69.0	84.0	75.0	66.8	79.5
	3	42.0	48.5	48.5	53.0	45.3	50.8
	4	43.5	42.0	58.5	48.5	42.8	53.5
	5	39.0	41.0	43.0	41.5	40.0	42.3
	6	46.0	43.0	58.5	53.0	44.5	55.8
	7	69.5	58.5	75.0	64.0	64.0	69.5
	8	84.0	75.0	94.0	97.5	79.5	95.8
10	1	6.1	4.8	7.7	6.3	5.4	7.0
	2	1.5	3.3	4.6	4.9	2.4	4.7
	3	3.3	1.5	6.2	3.3	2.4	4.7
	4	1.5	1.5	1.5	3.3	1.5	2.4
	5	0.0	0.0	1.5	0.0	0.0	0.8
	6	0.0	3.0	3.0	3.3	1.5	3.2
	7	1.5	3.3	3.3	4.9	2.4	4.1
	8	1.5	3.3	1.8	3.6	2.4	2.7
20	1	4.8	3.3	4.8	4.9	4.1	4.8
	2	0.0	3.0	0.0	3.3	1.5	1.7
	3	3.0	1.5	3.3	1.5	2.3	2.4
	4	0.0	0.0	1.5	1.5	0.0	1.5
	5	0.0	0.0	0.0	0.0	0.0	0.0
	6	0.0	0.0	3.0	1.5	0.0	2.3
	7	1.5	1.8	3.0	3.3	1.7	3.2
	8	0.0	3.0	0.0	3.6	1.5	1.8
		F value for ABCD = 0.4431 (d.f. for ABCD = 14/48)				F value for ACD = 0.39 (d.f. for ACD = 14/48)	

Table 18 Mean Total Coliform MPN Values of Oysters Stored at -20°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method Official	B. Method Rapid	C x D.
0	1	100.8	84.5	92.6
	2	74.0	72.3	73.1
	3	45.3	50.8	48.0
	4	51.0	45.3	48.1
	5	41.0	41.3	41.2
	6	52.3	48.0	50.1
	7	72.3	61.3	66.8
	8	89.0	86.3	87.6
10	1	6.9	5.6	6.2
	2	3.0	4.1	3.6
	3	4.7	2.4	3.6
	4	1.5	2.4	2.0
	5	0.8	0.0	0.4
	6	1.5	3.2	2.3
	7	2.4	4.1	3.3
	8	1.7	3.5	2.6
20	1	4.8	4.1	4.4
	2	0.0	3.2	1.6
	3	3.2	1.5	2.3
	4	0.8	0.8	0.8
	5	0.0	0.0	0.0
	6	1.5	0.8	1.1
	7	2.3	2.6	2.4
	8	0.0	3.3	1.7
		F value for BCD = 1.2160 (d.f. for BCD = 14/48)		F value for CD = 23.90** (d.f. for CD = 14/48)

**p < 0.01

Table 19 Mean Total Coliform MPN Values of Oysters Stored at -20°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	35.0	30.7	40.0	32.1
2	October	21.8	25.3	29.5	27.7
3	November	16.1	17.2	19.3	19.3
4	December	15.0	14.5	20.5	17.8
5	January	13.0	13.7	14.8	13.8
6	February	15.3	15.3	21.5	19.3
7	March	24.2	21.2	27.1	24.1
8	April	28.5	27.1	31.9	34.9

F value for ABD = 0.4327
(d.f. for ABD = 7/48)

Bivariate, A x D

	A. Treatment	
D. Mo.	Washed	Unwashed
1	32.8	36.1
2	23.6	28.6
3	16.6	19.3
4	14.8	19.1
5	13.3	14.3
6	15.3	20.4
7	22.7	25.6
8	27.8	33.4

F value for AD = 0.38
(d.f. for AD = 7/48)

Bivariate, B x D

	B. Method	
	Official	Rapid
1	37.5	31.4
2	25.7	26.5
3	17.7	18.2
4	17.8	16.1
5	13.9	13.8
6	18.4	17.3
7	25.6	22.6
8	30.7	31.0

F value for BD = 1.2103
(d.f. for BD = 7/48)

Harvest Month Means - D
34.4
26.1
18.0
16.9
13.8
17.9
24.1
30.6

F value for D = 33.10**
(d.f. for D = 7/48)

**p < 0.01

Table 20 Mean Total Coliform MPN Values of Oysters Stored at -20°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	10	20	
Washed	Official	60.3	1.9	1.2	21.1
	Rapid	57.7	2.6	1.6	20.6
Unwashed	Official	71.2	3.7	2.0	25.6
	Rapid	64.7	3.7	2.4	23.6
		F value for ABC = 0.6351 (d.f. for ABC = 2/48)			F value for AB = 0.9434 (d.f. for AB = 1/48)
Bivariate Interaction, A x C					**A. Treatment Means**
A. Treatment					
Washed		59.0	2.3	1.4	20.9
Unwashed		67.9	3.7	2.2	24.5
		F value for AC = 8.49** (d.f. for AC = 2/48)			F value for A = 17.39** (d.f. for A = 1/48)
Bivariate Interaction, B x C					**B. Method Means**
B. Method					
Official		65.7	2.8	1.6	23.3
Rapid		61.2	3.1	2.0	22.1
		F value for BC = 4.5816* (d.f. for BC = 2/48)			F value for B = 2.6295 (d.f. for B = 1/48)
C. Elapsed Time Means		63.4	3.0	1.8	Overall Mean 22.7
		F value for C = 2,069.11** (d.f. for C = 2/48)			

**p < 0.01
*p < 0.05

Table 21 Analysis of Variance for *Escherichia coli* MPN Values of Oysters Stored at 0°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C - Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	118.92	66.5769**
C	2	28.96	16.2159**
D	7	237.00	132.6908**
AC	2	2.53	1.419
AD	7	3.28	1.8350
CD	14	7.71	4.3161
ACD	14	2.62	1.4683
Error Term for Main Plots: Dup (ACD)	48	1.7861	
Subplots			
B	1	0.67	0.4825
AB	1	0.02	0.0136
BC	2	0.96	0.6948
BD	7	3.79	2.7508*
ABC	2	0.16	0.1185
ABD	7	2.38	1.7280
BCD	14	1.44	1.0433
ABCD	14	1.69	1.2272
Residual	14	1.38	

**$p < 0.01$
*$p < 0.05$

Table 22 Mean *Escherichia coli* MPN Values of Oysters Stored at 0°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate, A x C x D	
C.	D.	Washed		Unwashed			
Day	Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	12.0	10.2	12.0	13.0	11.1	12.5
	2	9.1	9.4	10.0	9.4	9.2	9.7
	3	6.7	6.7	7.2	8.1	6.7	7.7
	4	3.0	3.3	5.4	3.6	3.2	4.5
	5	3.0	3.6	3.3	4.9	3.3	4.1
	6	4.5	3.0	6.7	4.9	3.8	5.8
	7	9.2	8.2	12.0	9.6	8.7	10.8
	8	9.1	9.1	9.2	9.1	9.1	9.2
7	1	9.1	7.2	9.3	12.0	8.1	10.6
	2	8.3	8.2	9.2	9.2	8.2	9.2
	3	8.1	8.1	9.1	8.1	8.1	8.6
	4	3.0	1.8	6.7	3.3	2.4	5.0
	5	1.5	3.6	3.3	3.6	2.6	3.5
	6	1.5	1.5	4.5	3.3	1.5	3.9
	7	10.0	9.2	10.0	10.6	9.6	10.3
	8	3.3	6.0	9.3	10.0	4.7	9.7
14	1	9.1	9.1	9.3	9.3	9.1	9.3
	2	9.1	10.2	9.4	10.7	9.8	10.0
	3	4.6	3.0	7.7	5.4	3.8	6.5
	4	0.0	0.0	3.1	3.0	0.0	3.0
	5	1.5	3.0	3.0	3.6	2.3	3.3
	6	3.0	0.0	3.0	4.8	1.5	3.9
	7	10.2	11.0	11.5	11.0	10.6	11.3
	8	4.8	6.1	8.1	8.3	5.4	8.2
		F value for ABCD = 1.2272 (d.f. for ABCD = 14/48)				F value for ACD = 1.4683 (d.f. for ACD = 14/48)	

Table 23 Mean *Escherichia coli* MPN Values of Oysters Stored at 0°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C.	D.	B. Method		
Day	Mo.	Official	Rapid	C x D.
0	1	12.0	11.6	11.8
	2	9.6	9.4	9.5
	3	6.9	7.4	7.2
	4	4.2	3.5	3.8
	5	3.2	4.2	3.7
	6	5.6	4.0	4.8
	7	10.6	8.9	9.8
	8	9.2	9.1	9.1
7	1	9.2	9.6	9.4
	2	8.7	8.7	8.7
	3	8.6	8.1	8.3
	4	4.8	2.6	3.7
	5	2.4	3.6	3.0
	6	3.0	2.4	2.7
	7	10.0	9.9	9.9
	8	6.3	8.0	7.2
14	1	9.2	9.2	9.2
	2	9.4	10.4	9.9
	3	6.1	4.2	5.2
	4	1.5	1.5	1.5
	5	2.3	3.3	2.8
	6	3.0	2.4	2.7
	7	10.8	11.0	10.9
	8	6.5	7.2	6.8
		F Value for BCD = 1.0433 (d.f. for BCD = 14/48)		F value for CD = 4.3161 (d.f. for CD = 14/48)

Table 24 Mean *Escherichia coli* MPN Values of Oysters Stored at 0°C for ABD, AD, BD, and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	10.0	8.8	10.2	11.4
2	October	8.9	9.2	9.5	9.8
3	November	6.4	5.9	8.0	7.2
4	December	2.0	1.7	5.0	3.3
5	January	2.0	3.4	3.2	4.0
6	February	3.0	1.5	4.7	4.3
7	March	9.8	9.5	11.2	10.4
8	April	5.7	7.0	8.9	9.1

F value for ABD = 1.7280
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment: Washed	Unwashed
1	9.4	10.8
2	9.1	9.6
3	6.2	7.6
4	1.9	4.2
5	2.7	3.6
6	2.3	4.5
7	9.6	10.8
8	6.4	9.0

F value for AD = 1.8350
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method: Official	Rapid
10.1	10.1
9.2	9.5
7.2	6.6
3.5	2.5
2.6	3.7
3.9	2.9
10.5	9.9
7.3	8.1

F value for BD = 2.7508*
(d.f. for BD = 7/48)

Harvest Month Means - D
10.1
9.4
6.9
3.0
3.2
3.9
10.2
7.7

F value for D = 132.6908**
(d.f. for D = 7/48)

**p < 0.01
*p < 0.05

Table 25 Mean Escherichia coli MPN Values of Oysters Stored at 0°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	7	14	
Washed	Official	7.1	5.6	5.3	6.0
	Rapid	6.7	5.7	5.3	5.9
Unwashed	Official	8.2	7.7	6.9	7.6
	Rapid	7.8	7.5	7.0	7.4
		F value for ABC = 0.1185 (d.f. for ABC = 2/48)			F value for AB 0.0136 (d.f. for AB = 1/48)

Bivariate Interaction, A x C				A. Treatment Means
A. Treatment				
Washed	6.9	5.6	5.3	5.9
Unwashed	8.0	7.6	6.9	7.5
	F value for AC = 1.419 (d.f. for AC = 2/48)			F value for A = 66.5769** (d.f. for A = 1/48)

Bivariate Interaction, B x C				B. Method Means
B. Method				
Official	7.6	6.6	6.1	6.8
Rapid	7.3	6.6	6.1	6.7
	F value for BC = 0.6948 (d.f. for BC = 2/48)			F value for B = 0.4825 (d.f. for B = 1/48)

C. Elapsed Time Means	7.4	6.6	6.1	Overall Mean 6.7
	F value for C = 16.2159** (d.f. for C = 2/48)			

**p < 0.01

Table 26 Analysis of Variance for Escherichia coli MPN Values of Oysters Stored at -20°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	104.28	64.2171**
C	2	746.49	459.6898**
D	7	63.48	39.0926**
AC	2	2.44	1.5022
AD	7	1.61	0.9952
CD	14	15.24	9.3873
ACD	14	0.65	0.3989
Error Term for Main Plots: Dup (ACD)	48	1.62	
Subplots			
B	1	0.39	0.2376
AB	1	0.25	0.1494
BC	2	3.00	1.8090
BD	7	1.49	0.8968
ABC	2	0.11	0.0679
ABD	7	1.94	1.1692
BCD	14	2.60	1.5668
ABCD	14	1.46	0.8821
Residual	48	1.66	

**p < 0.01

Table 27. Mean Escherichia coli MPN Values of Oysters Stored at -20°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

C. Day	D. Mo.	Quadrivariate Interaction, A x B x C x D. Washed Official	Washed Rapid	Unwashed Official	Unwashed Rapid	Trivariate, A x C x D Washed	Trivariate, A x C x D Unwashed
0	1	12.0	10.2	12.0	13.0	11.1	12.5
	2	9.1	9.4	10.0	9.4	9.3	9.7
	3	6.7	6.7	7.2	8.1	6.7	7.7
	4	3.0	3.3	5.4	3.6	3.2	4.5
	5	3.0	3.6	3.3	4.9	3.3	4.1
	6	4.5	3.0	6.7	4.9	3.8	5.8
	7	9.2	8.2	12.0	9.6	8.7	10.8
	8	9.1	9.1	9.2	9.1	9.1	9.2
10	1	3.3	3.6	4.6	5.4	3.5	5.0
	2	1.5	0.0	3.6	3.6	0.8	3.6
	3	3.0	1.5	4.6	3.3	2.3	3.9
	4	0.0	0.0	0.0	3.0	0.0	1.5
	5	0.0	0.0	3.0	0.0	0.0	1.5
	6	0.0	0.0	1.5	3.0	0.0	2.3
	7	1.5	3.3	4.6	4.9	2.4	4.7
	8	0.0	1.5	1.5	3.3	0.8	2.4
20	1	0.0	1.5	1.5	3.3	0.8	2.4
	2	1.5	0.0	1.5	3.3	0.8	2.4
	3	0.0	1.5	1.5	3.0	0.8	2.3
	4	0.0	0.0	0.0	1.5	0.0	0.8
	5	0.0	0.0	1.5	0.0	0.0	0.8
	6	0.0	0.0	1.5	0.0	0.0	0.8
	7	0.0	1.5	4.5	3.3	0.8	3.9
	8	0.0	0.0	0.0	1.5	0.0	0.8
		F value for ABCD = 0.8821 (d.f. for ABCD = 14/48)				F value for ACD = 0.3989 (d.f. for ACD = 14/48)	

Table 28 Mean *Escherichia coli* MPN Values of Oysters Stored at -20°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	12.0	11.6	11.8
	2	9.6	9.4	9.5
	3	6.9	7.4	7.2
	4	4.2	3.5	3.8
	5	3.2	4.3	3.7
	6	5.6	4.0	4.8
	7	10.6	8.9	9.8
	8	9.2	9.1	9.1
10	1	3.9	4.5	4.2
	2	2.6	1.8	2.1
	3	3.8	2.4	3.1
	4	0.0	1.5	0.8
	5	1.5	0.0	0.8
	6	0.8	1.5	1.1
	7	3.0	4.1	3.6
	8	0.8	2.4	1.6
20	1	0.8	2.4	1.6
	2	1.5	1.7	1.6
	3	0.8	2.3	1.5
	4	0.0	0.8	0.4
	5	0.8	0.0	0.4
	6	0.8	0.0	0.4
	7	2.3	2.4	2.3
	8	0.0	0.8	0.4
		F value for BCD = 1.5668 (d.f. value for BCD = 14/48)		F value for CD = 9.3873 (d.f. for CD = 14/148

Table 29 Mean *Escherichia coli* MPN Values of Oysters Stored at -20°C for ABD, AD, BD, and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	5.1	5.1	6.0	7.2
2	October	4.0	3.1	5.0	5.4
3	November	3.2	3.2	4.4	4.8
4	December	1.0	1.1	1.8	2.7
5	January	1.0	1.2	2.6	1.6
6	February	1.5	1.0	3.2	2.6
7	March	3.6	4.3	7.0	5.9
8	April	3.0	3.5	3.6	4.6

F value for ABD = 1.1692
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment: Washed	A. Treatment: Unwashed
1	5.1	6.6
2	3.6	5.2
3	3.2	4.6
4	1.1	2.3
5	1.1	2.1
6	1.3	3.0
7	4.0	6.5
8	3.3	4.1

F value for AD = 0.9952
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method: Official	B. Method: Rapid
5.6	6.2
4.5	4.3
3.8	4.0
1.4	1.9
1.8	1.4
2.4	1.8
5.3	5.1
3.3	4.1

F value for BD = 0.8968
(d.f. for BD = 7/48)

Harvest Month Means - D
5.9
4.4
3.9
1.7
1.6
2.1
5.2
3.7

F value for D = 39.0926**
(d.f. for D = 7/48)

**p < 0.01

Table 30 Mean *Escherichia coli* MPN Values of Oysters Stored at -20°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	10	20	
Washed	Official	7.1	1.2	0.2	2.8
	Rapid	6.7	1.2	0.6	2.8
Unwashed	Official	8.2	2.9	1.5	4.2
	Rapid	7.8	3.3	2.0	4.4
		F value for ABC = 0.0679 (d.f. for ABC = 2/48)			F value for AB = 0.1494 (d.f. for AB = 1/48)
Bivariate Interaction, A x C					A. Treatment Means
A. Treatment					
Washed		6.9	1.2	0.4	2.8
Unwashed		8.0	3.1	1.7	4.3
		F value for AC = 1.5022 (d.f. for AC = 2/48)			F value for A = 64.2171** (d.f. for A = 1/48)
Bivariate Interaction, B x C					B. Method Means
B. Method					
Official		7.6	2.0	0.8	3.5
Rapid		7.2	2.3	1.3	3.6
		F value for BC = 1.8090 (d.f. for BC = 2/48)			F value for B = 0.2376 (d.f. for B = 1/48)
C. Elapsed Time Means		7.4	2.2	1.1	Overall Mean 3.6
		F value for C = 459.6898** (d.f. for C = 2/48)			

**p < 0.01

Table 31 Analysis of Variance for Coagulase-Positive Staphylococci MPN Values of Oysters Stored at 0°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	171.76	77.4436**
C	2	23.24	10.4790**
D	7	16.52	7.4505**
AC	2	5.30	2.3900
AD	7	3.15	1.4204
CD	14	1.47	0.6622
ACD	14	0.40	0.1790
Error Term for Main Plots: Dup (ACD)	48	2.2179	
Subplots			
B	1	0.0352	0.0188
AB	1	4.2602	2.2731
BC	2	1.9308	1.0302
BD	7	5.1962	2.7725*
ABC	2	1.3258	0.7074
ABD	7	6.1297	3.2706**
BCD	14	0.9321	0.4974
ABCD	14	0.6271	0.3346
Residual	48	1.8742	

**p < 0.01
*p < 0.05

Table 32 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at 0°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate, A x C x D	
		Washed		Unwashed			
C. Day	D. Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	0.0	3.3	6.1	4.5	1.7	5.3
	2	0.0	0.0	3.1	1.5	0.0	2.3
	3	0.0	0.0	0.0	1.5	0.0	0.8
	4	1.5	3.3	6.1	4.9	2.4	5.5
	5	1.5	1.5	3.0	3.0	1.5	3.0
	6	1.8	0.0	3.3	4.6	0.9	3.9
	7	0.0	0.0	4.9	1.5	0.0	3.2
	8	1.5	0.0	4.6	1.8	0.8	3.2
7	1	0.0	1.5	3.3	3.0	0.8	3.2
	2	0.0	0.0	3.1	0.0	0.0	1.5
	3	0.0	0.0	0.0	1.5	0.0	0.8
	4	0.0	3.0	4.5	4.9	1.5	4.7
	5	0.0	1.5	1.5	3.3	0.8	2.4
	6	1.5	0.0	1.5	3.0	0.8	2.3
	7	0.0	0.0	3.3	1.5	0.0	2.4
	8	0.0	0.0	1.5	1.5	0.0	1.5
14	1	0.0	1.5	1.5	3.0	0.8	2.3
	2	0.0	0.0	3.0	0.0	0.0	1.5
	3	0.0	0.0	0.0	1.5	0.0	0.8
	4	0.0	1.5	3.3	3.0	0.8	3.2
	5	0.0	0.0	0.0	1.5	0.0	0.8
	6	1.5	0.0	1.5	1.5	0.8	1.5
	7	0.0	0.0	3.0	1.5	0.0	2.3
	8	0.0	0.0	0.0	1.5	0.0	0.8
		F value for ABCD = 0.3346 (d.f. for ABCD = 14/48)				F value for ACD = 0.1790 (d.f. for ACD = 14/48)	

Table 33 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at 0°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	3.0	3.9	3.5
	2	1.5	0.8	1.1
	3	0.0	0.8	0.4
	4	3.8	4.1	3.9
	5	2.3	2.3	2.3
	6	2.6	2.3	2.4
	7	2.4	0.8	1.6
	8	3.0	0.9	2.0
7	1	1.7	2.3	2.0
	2	1.5	0.0	0.8
	3	0.0	0.8	0.4
	4	2.3	3.9	3.1
	5	0.8	2.4	1.6
	6	1.5	1.5	1.5
	7	1.7	0.8	1.2
	8	0.8	0.8	0.8
14	1	0.8	2.3	1.5
	2	1.5	0.0	0.8
	3	0.0	0.8	0.4
	4	1.7	2.3	2.0
	5	0.0	0.8	0.4
	6	1.5	0.8	1.1
	7	1.5	0.8	1.1
	8	0.0	0.8	0.4
		F value for BCD = 0.4974 (d.f. for BCD = 14/48)		F value for CD = 0.6622 (d.f. for CD = 14/48

Table 34 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at 0°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	0.0	2.1	3.6	3.5
2	October	0.0	0.0	3.0	0.5
3	November	0.0	0.0	0.0	1.5
4	December	0.5	2.6	4.6	4.2
5	January	0.5	1.0	1.5	2.6
6	February	1.6	0.0	2.1	3.0
7	March	0.0	0.0	3.7	1.5
8	April	0.5	0.0	2.0	1.6

F value for ABD = 3.2706**
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment Washed	A. Treatment Unwashed
1	1.1	3.6
2	0.0	1.8
3	0.0	0.8
4	1.6	4.4
5	0.8	2.1
6	0.8	2.6
7	0.0	2.6
8	0.3	1.8

F value for AD = 1.4204
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method Official	B. Method Rapid
1.8	2.8
1.5	0.3
0.0	0.8
2.6	3.4
1.0	1.8
1.9	1.5
1.9	0.8
1.3	0.8

F value for BD = 2.7725*
(d.f. for BD = 7/48)

Harvest Month Means - D
2.3
0.9
0.4
3.0
1.4
1.6
1.3
1.0

F value for D = 7.4505**
(d.f. for D = 7/48)

**p < 0.01
*p < 0.05

Table 35 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at 0°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days)			
		0	7	14	
Washed	Official	0.8	0.2	0.2	0.4
	Rapid	1.0	0.8	0.4	0.7
Unwashed	Official	3.9	2.3	1.5	2.6
	Rapid	2.9	2.3	1.7	2.3
		F value for ABC = 0.7074 (d.f. for ABC = 2/48)			F value for AB = 2.731 (d.f. for AB = 1/48)

Bivariate Interaction, A x C				A. Treatment Means
A. Treatment				
Washed	0.9	0.5	0.3	0.6
Unwashed	3.4	2.3	1.6	2.4
	F value for AC = 2.3900 (d.f. for AC = 2/48)			F value for A = 77.4436** (d.f. for A = 1/48)

Bivariate Interaction, B x C				B. Method Means
B. Method				
Official	2.3	1.3	0.9	1.5
Rapid	2.0	1.5	1.0	1.5
	F value for BC = 1.0302 (d.f. for BC = 2/48)			F value for B = 0.0188 (d.f. for B = 1/48)

C. Elapsed Time Means				Overall Mean	
	2.1	1.4	0.9		1.5
	F value for C = 10.4790** (d.f. for C = 2/48)				

**p < 0.01

Table 36 Analysis of Variance for Coagulase-Positive Staphylococci Values of Oysters Stored at -20°C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month

Source of Variation	d. f.	M. S.	F.
Total	191		
Main Plots			
A	1	50.02	36.6705**
C	2	78.62	57.6341**
D	7	5.28	3.8742**
AC	2	26.95	19.7537**
AD	7	0.99	0.7232
CD	14	4.01	2.9381**
ACD	14	0.75	0.5509
Error Term for Main Plots: Dup (ACD)	48	1.3641	
Subplots			
B	1	0.0033	0.0025
AB	1	0.8008	0.6037
BC	2	2.3265	1.7538
BD	7	0.8570	0.6461
ABC	2	2.7602	2.0807
ABD	7	2.6245	1.9784
BCD	14	1.2280	0.9257
ABCD	14	1.0739	0.8095
Residual	48	1.3266	

**p < 0.01

Table 37 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at -20°C for ABCD and ACD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

		Quadrivariate Interaction, A x B x C x D.				Trivariate, A x C x D	
C.	D.	Washed		Unwashed			
Day	Mo.	Official	Rapid	Official	Rapid	Washed	Unwashed
0	1	0.0	3.3	6.1	4.5	1.7	5.3
	2	0.0	0.0	3.1	1.5	0.0	2.3
	3	0.0	0.0	0.0	1.5	0.0	0.8
	4	1.5	3.3	6.1	4.9	2.4	5.5
	5	1.5	1.5	3.0	3.0	1.5	3.0
	6	1.8	0.0	3.3	4.6	0.9	3.9
	7	0.0	0.0	4.9	1.5	0.0	3.2
	8	1.5	0.0	4.6	1.8	0.8	3.2
10	1	0.0	0.0	1.5	0.0	0.0	0.8
	2	0.0	1.5	1.5	1.5	0.8	1.5
	3	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	1.5	0.0	1.5	0.8	0.8
	5	0.0	0.0	0.0	1.5	0.0	0.8
	6	1.5	0.0	1.5	3.3	0.8	2.4
	7	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	1.5	0.0	0.8
20	1	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0
	5	0.0	0.0	0.0	0.0	0.0	0.0
	6	0.0	0.0	0.0	0.0	0.0	0.0
	7	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.0	0.0	0.0	0.0	0.0	0.0
		F value for ABCD = 0.8095 (d.f. for ABCD = 14/48)				F value for ACD = 0.5509 (d.f. for ACD = 14/48)	

Table 38 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at -20°C for BCD and CD

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, B x C x D.				Bivariate
C. Day	D. Mo.	B. Method: Official	B. Method: Rapid	C x D.
0	1	3.0	3.9	3.5
	2	1.5	0.8	1.1
	3	0.0	0.8	0.4
	4	3.8	4.1	3.9
	5	2.3	2.3	2.3
	6	2.6	2.3	2.4
	7	2.4	0.8	1.6
	8	3.0	0.9	2.0
10	1	0.8	0.0	0.4
	2	0.8	1.5	1.1
	3	0.0	0.0	0.0
	4	0.0	1.5	0.8
	5	0.0	0.8	0.4
	6	1.5	1.7	1.6
	7	0.0	0.0	0.0
	8	0.0	0.8	0.4
20	1	0.0	0.0	0.0
	2	0.0	0.0	0.0
	3	0.0	0.0	0.0
	4	0.0	0.0	0.0
	5	0.0	0.0	0.0
	6	0.0	0.0	0.0
	7	0.0	0.0	0.0
	8	0.0	0.0	0.0
		F value for BCD = 0.9257 (d.f. for BCD = 14/48)		F value for CD = 2.9381** (d.f. for CD = 14/48

**p < 0.01

Table 39 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at -20°C for ABD, AD, BD and D

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days); D = Harvest Month (1-8: September, 1975 - April, 1976)

Trivariate Interaction, A x B x D

A. Treatment:		Washed		Unwashed	
B. Method:		Official	Rapid	Official	Rapid
D.	Month				
1	September	0.0	1.1	2.5	1.5
2	October	0.0	0.5	1.5	1.0
3	November	0.0	0.0	0.0	0.5
4	December	0.5	1.6	2.0	2.1
5	January	0.5	0.5	1.0	1.5
6	February	1.1	0.0	1.6	2.6
7	March	0.0	0.0	1.6	0.5
8	April	0.5	0.0	1.5	1.1

F value for ABD = 1.9784
(d.f. for ABD = 7/48)

Bivariate, A x D

D. Mo.	A. Treatment: Washed	Unwashed
1	0.6	2.0
2	0.3	1.3
3	0.0	0.3
4	1.1	2.1
5	0.5	1.3
6	0.6	2.1
7	0.0	1.1
8	0.3	1.3

F value for AD = 0.7232
(d.f. for AD = 7/48)

Bivariate, B x D

B. Method: Official	Rapid
1.3	1.3
0.8	0.8
0.0	0.3
1.3	1.9
0.8	1.0
1.4	1.3
0.8	0.3
1.0	0.6

F value for BD = 0.6461
(d.f. for BD = 7/48)

Harvest Month Means - D
1.3
0.8
0.1
1.6
0.9
1.3
0.5
0.8

F value for D = 3.8742**
(d.f. for D = 7/48)

**p < 0.01

Table 40 Mean Coagulase-Positive Staphylococci MPN Values of Oysters Stored at -20°C for ABC, AB, AC, BC, A, B, and C

Variables: A = Washing Treatment; B = Laboratory Methodology; C = Elapsed Storage Time (Days)

Trivariate Interaction, A x B x C					Bivariate A x B
A. Treatment	B. Method	C. Elapsed Storage Time (Days) 0	10	20	
Washed	Official	0.8	0.2	0.0	0.3
	Rapid	1.0	0.4	0.0	0.5
Unwashed	Official	3.9	0.6	0.0	1.5
	Rapid	2.9	1.2	0.0	1.4
		F value for ABC = 2.0807 (d.f. for ABC = 2/48)			F value for AB = 0.6037 (d.f. for AB = 1/48)

Bivariate Interaction, A x C				A. Treatment Means
A. Treatment				
Washed	0.9	0.3	0.0	0.4
Unwashed	3.4	0.9	0.0	1.4
	F value for AC = 19.7537** (d.f. for AC = 2/48)			F value for A = 36.6705 (d.f. for A = 1/48)

Bivariate Interaction, B x C				B. Method Means
B. Method				
Official	2.3	0.4	0.0	0.9
Rapid	2.0	0.8	0.0	0.9
	F value for BC = 1.7538 (d.f. for BC = 2/48)			F value for B = 0.0025 (d.f. for B = 1/48)

C. Elapsed Time Means				Overall Mean	
	2.1	0.6	0.0		0.9
	F value for C = 57.6341** (d.f. for C = 2/48)				

**p < 0.01

VITA

Neal Duane Singletary was born on November 14, 1950 in Baton Rouge, Louisiana. He was graduated from East Ascension High School in May of 1968.

He entered Southeastern Louisiana University in September of 1968 and completed requirements for a Bachelor of Science degree in May of 1972.

He was admitted to the graduate school of Southeastern Louisiana University in June of 1972. His major field of study was Microbiology. He completed requirements for the Master of Science degree in May of 1974.

In June of 1974 he was admitted to the graduate school of Louisiana State University. His major field of study is Food Science and he is presently a candidate for the degree of Doctorate of Philosophy in the Department of Food Science.

EXAMINATION AND THESIS REPORT

Candidate: Neal D. Singletary

Major Field: Food Science

Title of Thesis: Rapid Analytical Procedures and Varying Preservation Conditions for Assessing the Bacterial Quality of Oysters (Crassostrea virginica)

Approved:

Robert M. Grodner
Major Professor and Chairman

James L. Graynham
Dean of the Graduate School

EXAMINING COMMITTEE:

William H. James

Rama Rao

James E. Cutlidge

A. J. Novak

William F. McKnight

Date of Examination:

May 18, 1977